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Dithrocream®
dithranol BP

Now in two strengths

Dithrocream – the effective, *non-steroid* treatment for psoriasis – is now available in a new low strength formulation ideal for assessing the reaction and responsiveness of patients to dithranol therapy. It can also be used for treating mild conditions where a lower strength may be preferred.

Just like Dithrocream 0.25%, low strength Dithrocream 0.1% is formulated in a *true* vanishing cream base[1] which can be applied in small quantities until completely absorbed[2]. The problems of burning and staining, traditionally associated with the use of dithranol, are thus minimised, whilst clinical effectiveness is maintained[3].

The treatment of choice for psoriasis

Presentation
Dithrocream 0.1%: pale yellow aqueous cream containing 0.1% Dithranol B.P.
Dithrocream 0.25%: pale yellow aqueous cream containing 0.25% Dithranol B.P.

Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp.

Directions for use
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
Acute psoriasis; dithranol sensitivity.

Precautions
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Prices
Basic N.H.S. prices:
Dithrocream 0.1% 50gm tubes £1.65
Dithrocream 0.25% 50gm tubes £1.79

Product Licence Nos.
Dithrocream 0.1% PL 0173/0029
Dithrocream 0.25% PL 0173/0028

References
1. *British Medical Journal*, 1978, **2**, 206.
2. *General Practitioner*, 1978, June 2, 45.
3. *British Journal of Dermatology*. Accepted for publication.

Dithro-
cream
0·1%
for the treatment of chronic and sub-acute psoriasis including psoriasis of the scalp
Dithro-
cream
0·25%
for the treatment of chronic and sub-acute psoriasis including psoriasis of the scalp

FURTHER INFORMATION, INCLUDING DATA SHEET, IS AVAILABLE FROM

PUVA for Psoriasis

PUVA facilities for the treatment of psoriasis are now available to private patients who are referred by Consultant Dermatologists. The Waldmann PUVA 6000 unit, widely acknowledged as the best of its kind, is used. It allows wholebody treatment in the standing position. There are also Waldmann PUVA 180, 200 and 500 units for the treatment of hands, feet and other small areas.

A regime has been developed based on a London Teaching Hospital's experience and practice. The staff are professionally qualified and trained in the Photochemotherapy of psoriasis. Visits by Dermatologists are welcome by appointment.

An Honorary Medical Advisory Committee made up of four consultant dermatologists is supervising the professional standards and practice of the clinic. Dermatologists, who would like to know more about this facility, are asked in the first instance to contact:

The Physician-in-Charge,
Psoriasis UVA (Medical Referals Only) Ltd.,
20 Harley Street, LONDON W.1.
Telephone 01-580 0875

ACNE?

prescribe patient confidence

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When patients like these ask for your help remember that clinical reports show that acne responds to Quinoderm.

Available as Quinoderm
with **Hydrocortisone Cream 30G.** for initial treatment.
Quinoderm Cream 25G. for subsequent treatment.

* Practitioner, 1970, 204, 639

Quinoderm Hydrocortisone 30G.
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in an astringent cream base.

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Quinoderm

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BENOXYL® 20

Benzoyl peroxide lotion 20%

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lications: Cutaneous ulcers.

ministration: following the dressing rt, apply a protective ointment to the area bordering the ulcer to protect mal skin. Place dressings saturated Benoxyl 20 Lotion in intimate act with ulcer surfaces.

tra-indications, warnings: keep from mouth, eyes and other mucous membranes and avoid normal skin. Irritant dermatitis reported in 2.5% to 3% of cases. If undue skin irritation develops discontinue product use. May bleach coloured fabrics. FOR EXTERNAL USE ONLY.

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Canad. Med. Assoc. J. 1976, **115,** 1101 J. Dermatol. Surg. Oncol 1978 **4:** 1, 91 Cutis, 1978, *21,* 491 J. Dermatol. Surg. Oncol. 1978, **4:** 9, 701 Canad. Fam. Phys., 1976, **22,** 1301

Benoxyl is a registered trademark
Further information and Dressing Chart is available from

Stiefel Laboratories (UK) Ltd
Wellcroft Road, Slough, Berks. SL1 4A

One look at her Grandad used to give Lucy nightmares.

Dermatological conditions can cause an awful lot of misery.

Particularly when they are widespread.

Sufferers can lose complete confidence in themselves. And the reaction of others is not always kind.

In fact the social effects are perhaps worse than the physiological ones.

And selecting the right treatment is no laughing matter.

Take potent fluorinated steroids for example. There's no doubting their effectiveness, but current medical opinion is increasingly concerned about their side-effects.

And hydrocortisone isn't always the answer. Because what you gain in safety you can lose in effectiveness.

Locoid offers you a third choice.

Its safety record is well-known. Experimental and clinical studies[1,2,3] have shown that Locoid has a low capacity for side effects, but did you know that Locoid, in clinical terms, is as effective as the potent fluorinated steroids?[4,5,6,7] And as fast-acting[8,9]

Perhaps in suggesting you have three choices we're being a bit misleading.

When you think about it, surely there's only one choice. Locoid.

Hydrocortisone 17-butyrate 0.1%

Locoid

Rely on its strength, trust in its safety.

Full information available on request:
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Locoid is a trade mark for hydrocortisone 17-butyrate. P/L numbers: 0166/0058; 0166/0059; 0166/0060.
Use: Recommended in the treatment of conditions responsive to topical corticosteroids, eg, eczema, psoriasis and dermatitis.
C/I: In the presence of infection unless used with suitable chemotherapy. Large amounts and long duration in pregnancy and long-term therapy in infants.
Apply two to four times daily. 30g cream/ointment £1.43. 20ml lotion £1.53.
References: 1. Brit J Derm (1976) 95,566 2. Brit J Derm (1977) 97,505 3. Brit J Derm Suppl 12, Vol 94 (1976) 4. Brit J Clin Pract (1973) 27,177 5. Dermatologica (1976) 152 Suppl 209 6. Brit J Clin Pract (1972) 26,263 7. Brit J Derm (1970) 83,93 8. Dermatologica (1976) 152 Suppl 221 9. Brit J Derm (1973) 88,83

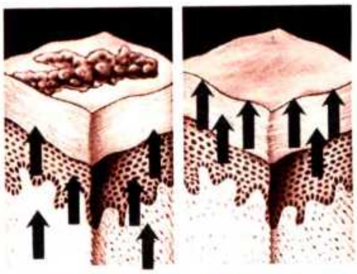
You can treat fungal infections

superficially. Or you can get to the root of the problem with Grisovin

Grisovin is griseofulvin, the oral antifungal antibiotic for systemic treatment of ringworm infections.

Proven in use for over 20 years, Grisovin offers all the benefits of oral over topical therapy: it is neither messy nor inconvenient to use; and for deep-seated conditions it is more effective. Most adult patients will need only one 500mg tablet twice a day. In addition, Grisovin is notable for its lack of side effects.

In the treatment of tinea infections, nail infections and scalp ringworm, the proven value of griseofulvin, its oral administration, unique action and dosage convenience, make Grisovin an effective, more acceptable therapy.



Unique action of Grisovin in skin.

1. Oral griseofulvin reaches basal layer via blood stream and protects newly formed cells from infection.
2. Outward movement of griseofulvin-protected keratin expels fungus.

Glaxo

Full prescribing information on Grisovin (trade mark) is available from:

Glaxo Laboratories Limited
Greenford, Middlesex UB6 0HE

GRISOVIN

griseofulvin

Prescribing information

Dosage and administration
Doses should be taken after meals for maximum absorption.
Adults: normally 500 to 1,000mg daily in divided doses, but not less than 10mg/kg bodyweight daily.
Children: usually 10mg per kg (5mg/lb) bodyweight daily in divided doses.
Duration of treatment: for hair or skin at least four weeks' treatment is required, whereas finger or toe nails may need six to twelve months' treatment. Continue for at least two weeks after all signs of infection have disappeared.

Contra-indications
Porphyria or severe liver disease. Griseofulvin may cause deterioration in some patients with liver disease and liver function should be monitored in such conditions.

Precautions
Griseofulvin may decrease the effect of coumarin anti-coagulants. Absorption is inhibited when phenobarbitone is taken concurrently. The blood level, and hence efficacy, may also be impaired as the result of concurrent administration of substances such as phenylbutazone and sedative and hypnotic drugs which induce metabolising enzymes. In pregnant animals, administration of very high doses of griseofulvin can cause foetal abnormalities. The relevance of this finding to humans has not been established.

Side effects
Headache, and gastric discomfort sometimes occur, but usually disappear as treatment continues. Rarely, urticarial reactions and erythematous rashes have been reported.

Further information
Grisovin is not effective in infections caused by *Candida albicans* (monilia), Aspergilli, *Malassezia furfur (Pityriasis versicolor)* and Nocardia species.

Presentation	**Product Licence number**
Tablets	
125mg (bottles of 100 and 1000)	4/5060
500mg (bottles of 25 and 100*)	4/5061

*Basic NHS cost (exclusive of VAT)— £7·14

This new journal replaces the British edition of the *Journal of the Society of Cosmetic Chemists* and is designed for the publication of original and review papers and correspondence in the wide fields of skin and cosmetic research. It is aimed at practising cosmetic scientists, dermatologists and those microbiologists, pharmacists, chemists and experimental biologists working on products which will come into contact with the human body. The aim of the journal is to provide an up-to-date presentation of current scientific research, both pure and applied, in cosmetics, toiletries, perfumery and allied fields. The specific interests include human and animal safety testing of skin, hair and oral products – physical chemistry and technology of emulsion and dispersed systems – theory and application of surface active agents – new developments in olfactive research – aerosol technology – selected aspects of analytic chemistry.

The *International Journal of Cosmetic Science* is published bi-monthly at £30.00 (U.K.), £36.00 (overseas), $80.00 (U.S.A. and Canada) per annum post free, including delivery by air to North America. Subscriptions and requests for specimen copies should be sent to Blackwell Scientific Publications Ltd, P.O. Box 88, Oxford, England

INTERNATIONAL JOURNAL OF COSMETIC SCIENCE

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Difficult Dermatoses

Dermovate

(clobetasol propionate)

Even long-standing, stubborn eczematous lesions respond quickly to Dermovate – with rapid relief from itching; and this is often followed by prolonged periods of remission. And because Dermovate is so rapidly effective, treatment need be of short duration only, minimising the risk of steroid side effects.

rescribing information

osage and administration

pply sparingly once or twice daily ntil improvement occurs. Treat- nent should not be continued for nore than four weeks without eview.

ide effects

Vith all topical corticosteroids cal atrophic changes may ssibly occur following prolonged d intensive treatment. Also pro- nged use of large amounts or eatment of extensive areas may oduce the features of hyper- rticism.

s is more likely to occur in nts and children, and with lusion. In infants, the napkin y act as an occlusive dressing. Provided the weekly dosage of Dermovate is less than 50 grams in adults, any pituitary-adrenal suppression is likely to be transient with a rapid return to normal values once the short course of steroid therapy has ceased. The same applies to children given proportionate dosage.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Rarely, treatment of psoriasis with corticosteroids (or its withdrawal) is thought to have provoked the pustular form of the disease.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion. The face, more than other areas of the body, may exhibit atrophic changes after prolonged treatment with potent topical corticosteroids.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Rosacea, acne and peri-oral dermatitis, all of which can be treated with oral tetracyclines. Viral diseases of the skin, e.g., herpes simplex.

Basic NHS cost (exclusive of VAT)

Dermovate Cream or Ointment 25 gram tubes £1·45 (also available in 100 gram tubes).

Presentation	**Product Licence numbe**
Dermovate Cream	4/0219
Ointment	4/0220

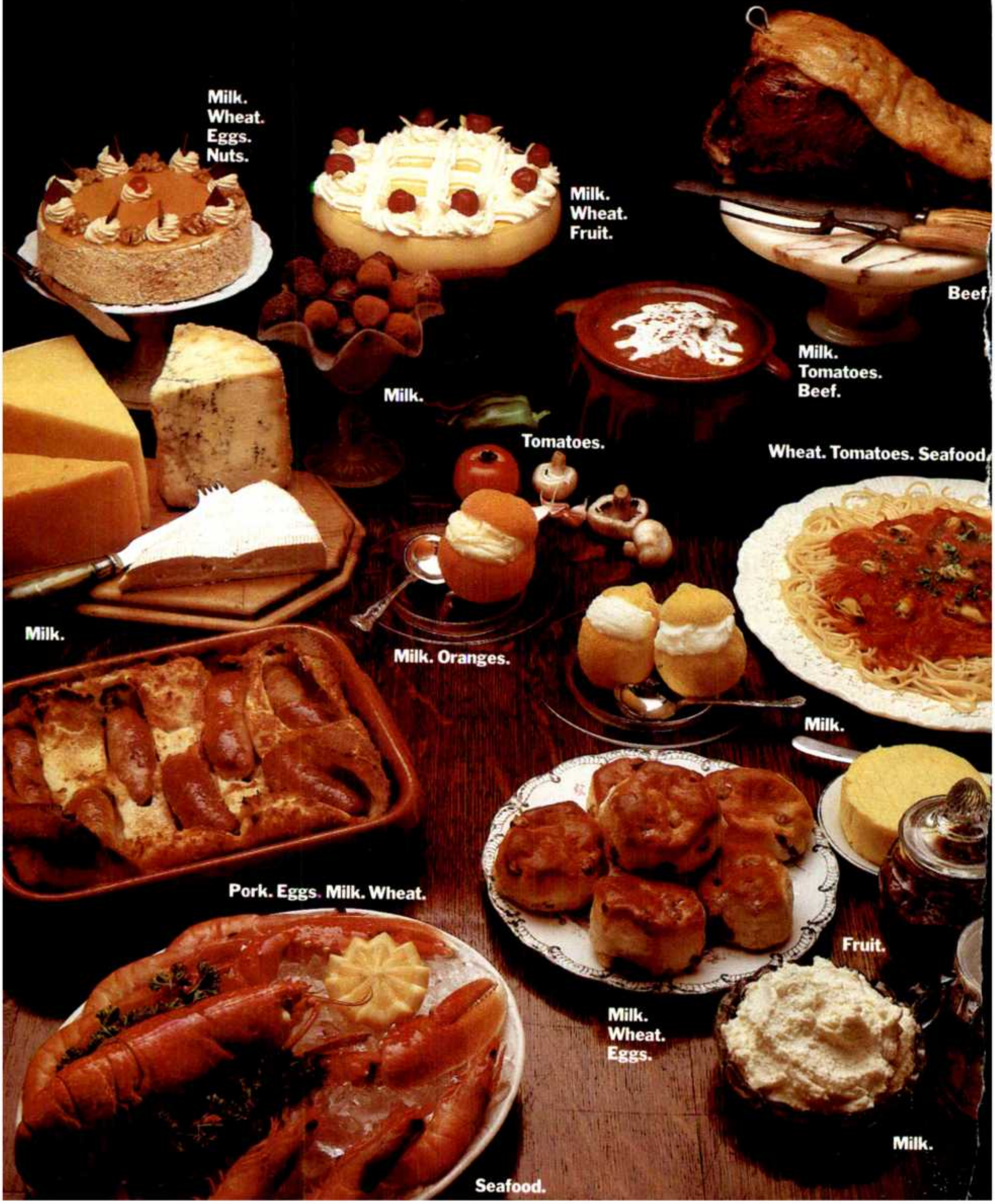
Glaxo

Leaders in topical steroid therapy

Further information on Dermovate (trade mark) is available from:

Glaxo Laboratories Ltd
Greenford, Middlesex UB6 0HE

Like it or not, food allergies do exist.



And so, at last, does an ffective drug treatment.

The whole business of food allergies dmittedly, a difficult, often unclear and etimes contentious one.

DIAGNOSIS: THE BASIC PROBLEM.

The symptoms of food allergies may ır in the gastro-intestinal tract, mimic ses in other systems.

Like, for example, chronic diarrhoea other gastro-intestinal symptoms), aria and eczema.

The exact mechanism is uncertain.

But it appears that initially the allergen es a reaction in the wall of the gut.

This, in turn, leads to gastro-intestinal ptoms or, indirectly, symptoms in other t' organs.

ELIMINATION DIETS: THE EASY ANSWER.

The obvious way to treat food allergies course, to eliminate offending foods.

It is no great hardship to be told to l eating things like tomatoes or oysters, all.

But the root of the problem can often ore complex.

And what can you do when, after stigation, the causes are such that total nation is impractical?

TRIALS AND RESULTS.

Studies involving 104 patients with allergy symptoms of eczema, urticaria, hoea or vomiting have been published.

The result?

Orally-administered sodium cromoglycate proved to be an effective treatment, for these difficult problems, in 59 patients (57%).

NALCROM. A TREATMENT.

Nalcrom is sodium cromoglycate.

Sodium cromoglycate – a drug with a history of over 10 years clinical efficacy and safety. It is, in fact, the only drug which can treat food allergies by prevention.

So, faced with a known allergy to a food which cannot easily be eliminated, why not add Nalcrom as a trial to the elimination diet?

Before you do, however, perhaps you would like a specialist representative to call on you to discuss the whole issue, and the way Nalcrom works.

Just post the coupon or write to Fisons Ltd, Pharmaceutical Division, Loughborough, Leicestershire, and let us arrange a convenient time.

Nalcrom®
SODIUM CROMOGLYCATE B.P.

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BJD 7

Fisons Limited, Pharmaceutical Division,
Derby Road, Loughborough, Leicestershire LE11 0BB.

ISONS Leaders in Allergy Research. Fisons Limited, Pharmaceutical Division, Derby Road, Loughborough, Leicestershire LE11 0BB. **Dosage and tration:** (a) Chronic inflammatory bowel disease as an adjuvant in the treatment of ulcerative colitis, proctitis and proctocolitis – two capsules four times dults and one capsule four times daily in children from 2-14 years, before meals. (b) Food allergy – as above for the initial dose. If satisfactory control is not within three weeks, the dosage may be doubled but not exceed 40mg/kg/day. Dosage may be reduced to the minimum required to maintain the patient free oms. Protection may be afforded by a single dose taken 15 minutes before a meal in which there may be an unavoidable allergenic food. The capsules may ved whole or the powder contents may be dissolved in hot water and diluted with cold water to drink. **Contra-indications:** There are no specific contra-s. **Warnings:** The safety in pregnancy and the treatment of children under two years has not yet been established. **Side effects:** Nausea, skin rashes and have been reported in a few cases. **Over-dosage:** No action other than medical observation should be necessary. **Basic NHS cost:** £17.14 per 100 capsules. 73.

® Registered Trade Mark.

International Coding Index for Dermatology

Compatible with the 9th Revision of the
WHO International Classification of Disease

Compiled by
Suzanne Alexander and Alan B. Shrank

This new index is designed to permit the accurate and efficient retrieval of information on dermatological diagnoses, and has a much wider scope than the index issued by the World Health Organization. The coding system is based upon the International Classification of Disease, which is understood and accepted universally, and it is entirely compatible with the Ninth Revision due to be inaugurated in 1979. It is compact yet inclusive of many new disorders, and will prove its value in epidemiological studies, in medical research and in the planning of health services. The coding system is becoming widely adopted throughout the world and dermatologists will therefore find this index to be increasingly useful in their practice.

1978. 112 pages. Paper, £2.00

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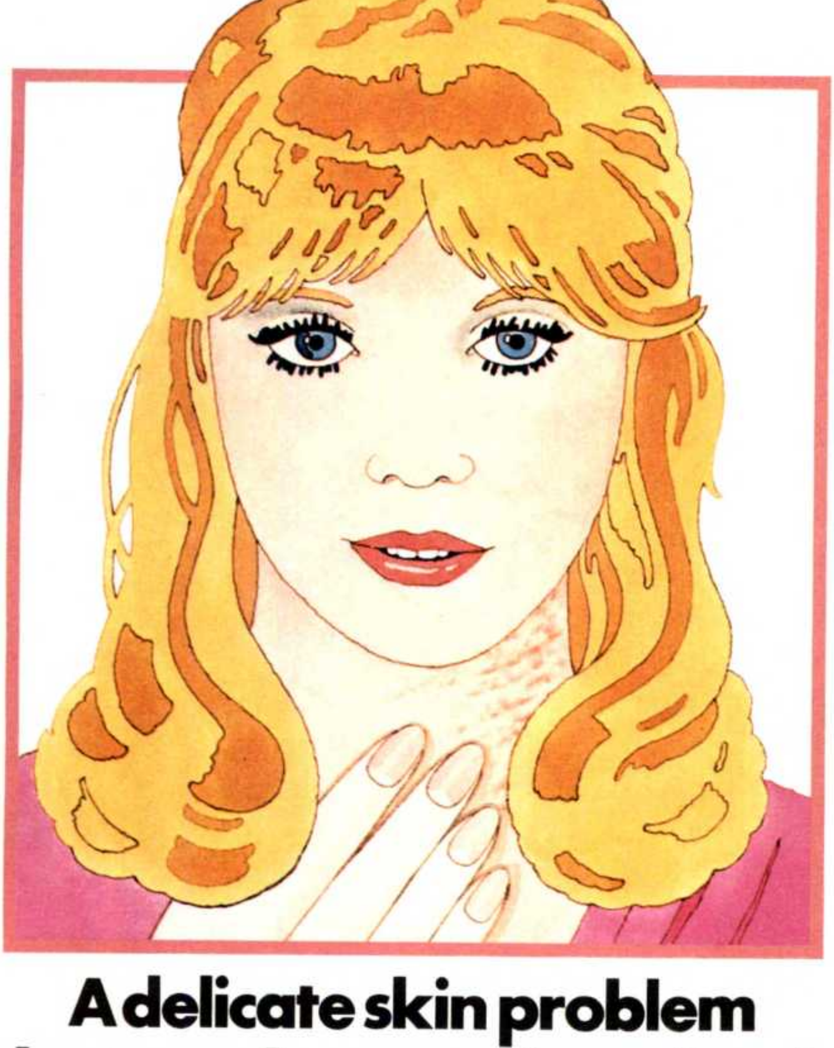

A delicate skin problem but one that must be solved

When prescribing a topical steroid to treat a delicate area, a major consideration is to avoid the risk of untoward effects.

Eumovate fulfils the need for a topical steroid with a wide margin of safety, providing significant anti-inflammatory activity without a corresponding increase in the risk of side effects.

Clinical evidence[1,2] has shown that the minimal effect on HPA function observed with Eumovate was in definite contrast to that seen with other preparations.

1. Munro, D.D., Wilson L.C., *British Medical Journal* (1975) **3,** 626
2. Munro, D.D., *Journal of Dermatology* (1976) **94** (Suppl.) 12 67

Eumovate

(clobetasone butyrate)

An investment in safety and efficacy

Prescribing information

Uses

Eumovate is suitable for treating the milder forms of eczema, seborrhoeic dermatitis and other steroid responsive skin conditions.

Dosage and administration

Apply up to four times a day until improvement occurs, when the frequency may be reduced.

Side effects

With all topical corticosteroids local atrophic changes may possibly occur following prolonged and intensive treatment. Also prolonged use of large amounts or treatment of extensive areas may produce the features of hypercorticism. This is more likely to occur in infants and children, and with occlusion. In infants, the napkin may act as an occlusive dressing.

In the unlikely event of signs of hypersensitivity appearing, application should stop immediately.

Precautions

Long-term continuous therapy should be avoided, particularly in infants and children in whom adrenal suppression can occur even without occlusion.

Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

With all corticosteroids, prolonged application to the face is undesirable.

Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Contra-indications

Bacterial, fungal or viral diseases of the skin.

Basic NHS cost (exclusive of VAT)

Eumovate Cream or Ointment 25 gram tube £1·23 (also available in 100 gram tubes)

Product Licence number

cream	ointment
4/0233	4/0254

Glaxo

Leaders in topical steroid therapy

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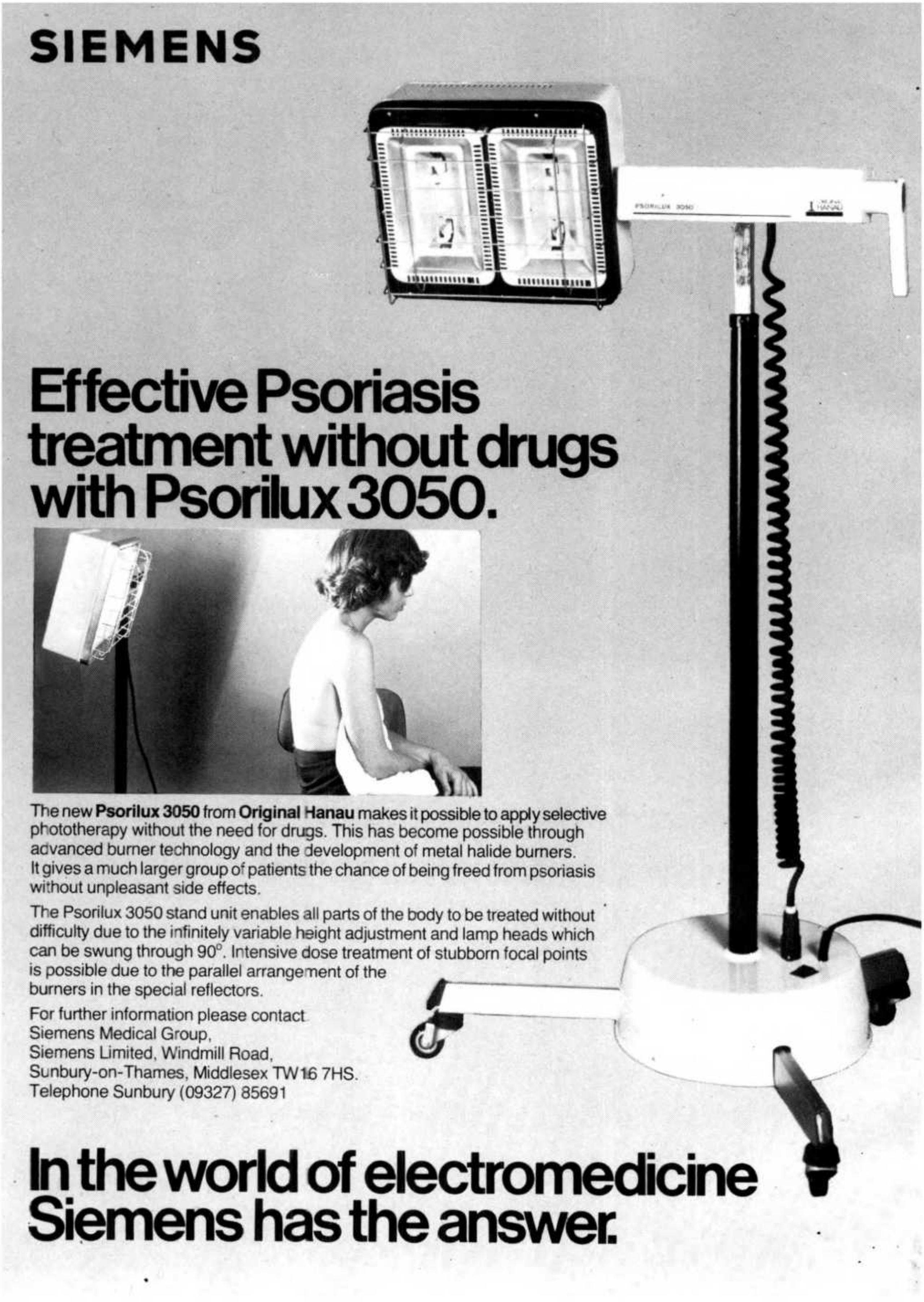
Greenford, Middlesex UB6 0HE
Eumovate is a trade mark



SIEMENS

Effective Psoriasis treatment without drugs with Psorilux 3050.

The new **Psorilux 3050** from **Original Hanau** makes it possible to apply selective phototherapy without the need for drugs. This has become possible through advanced burner technology and the development of metal halide burners. It gives a much larger group of patients the chance of being freed from psoriasis without unpleasant side effects.

The Psorilux 3050 stand unit enables all parts of the body to be treated without difficulty due to the infinitely variable height adjustment and lamp heads which can be swung through 90°. Intensive dose treatment of stubborn focal points is possible due to the parallel arrangement of the burners in the special reflectors.

For further information please contact
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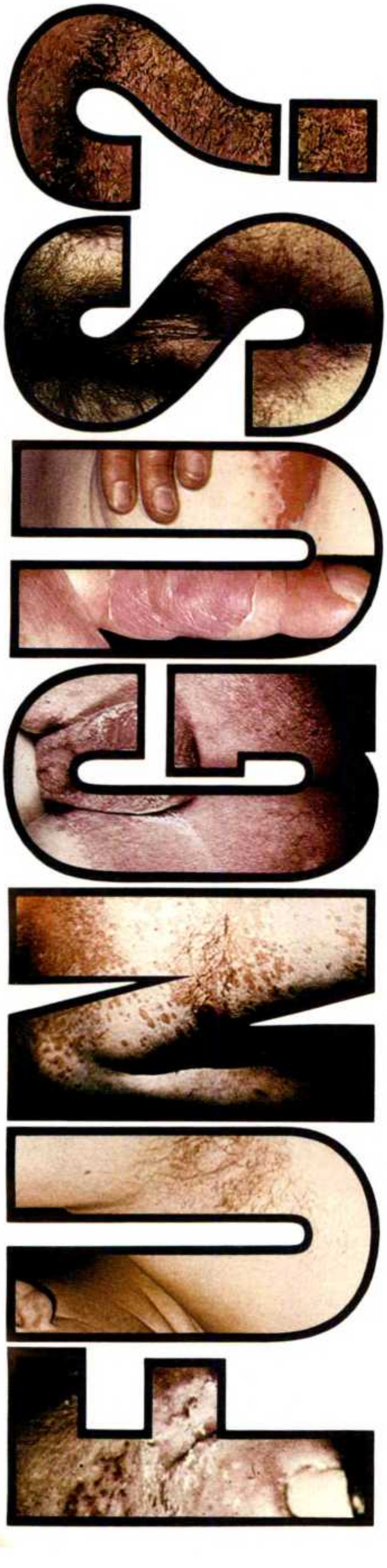
Telephone Sunbury (09327) 85691

In the world of electromedicine Siemens has the answer.



Canesten®

clotrimazole

Effective against all fungal skin infections

So effective treatment with Canesten may begin at once – without waiting for culture results.

Wherever on the body a fungal infection occurs, whatever the organism responsible, there is a need for speedy effective therapy. Canesten's broad range of presentations is the answer... cream, atomiser spray and now Canesten powder for adjunctive therapy and to help prevent re-infection.

Canesten

the most widely-prescribed antifungal

Prescribing Information

Presentations *Cream* A white cream containing 1.0% clotrimazole. *Atomiser Spray* A clear solution containing 1.0% clotrimazole in 30% isopropanol. *Powder* A white powder containing 1.0% clotrimazole.

Indications All fungal skin infections due to dermatophytes, yeasts, moulds and other fungi including ringworm (tinea) infections.

Dosage and Administration *Cream, Atomiser Spray* Apply to the affected area 2-3 times daily. *Powder* as above and may also be dusted inside articles of clothing and footwear which are in contact with the infected area as a prophylactic against re-infection.

Side-effects Rarely, patients may briefly experience a transient local mild burning or irritation.

Precautions Canesten atomiser spray should not be used near a naked flame, should not be allowed to come into contact with the eyes, ears or mucous membranes and should not be inhaled.

Product Licence Numbers Cream: PL 0010/0016. Atomiser Spray: PL 0010/0060. Powder: PL 0010/0067.

Basic NHS prices Cream: 20g, £1.37; 50g, £3.20. Atomiser Spray: 40ml, £3.68. Powder: 30g, £0.99.

Further information is available from
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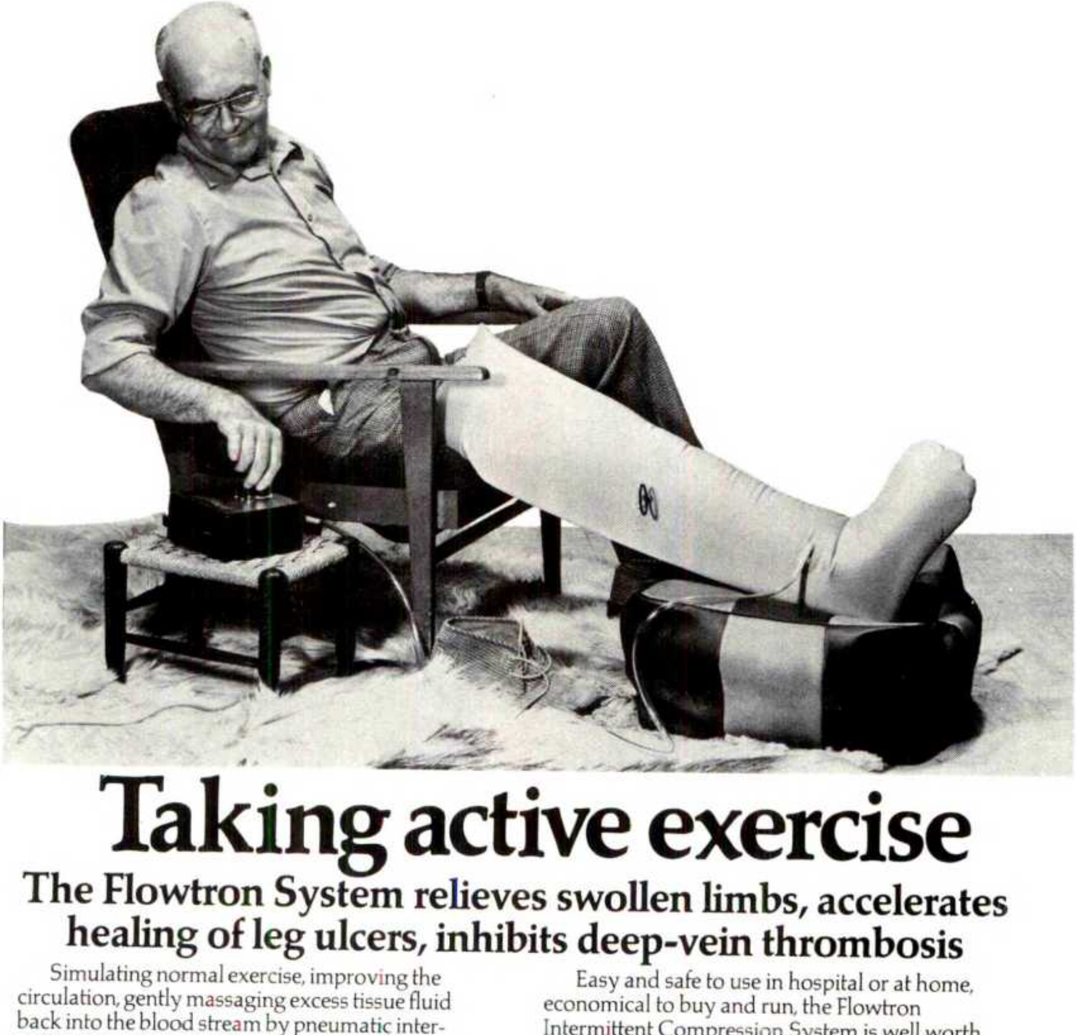

Taking active exercise

The Flowtron System relieves swollen limbs, accelerates healing of leg ulcers, inhibits deep-vein thrombosis

Simulating normal exercise, improving the circulation, gently massaging excess tissue fluid back into the blood stream by pneumatic intermittent compression. The non-invasive Flowtron System works by means of a silent running electric pump alternately inflating and deflating double-walled garments.

Case histories submitted by doctors and patients alike show dramatic reduction in swollen limbs, including post-mastectomy lymphoedema, and marked improvement in leg ulcers, through using the Flowtron System. In controlled trials, reductions of up to 90% in the incidence of deep-vein thrombosis have been noted.

Easy and safe to use in hospital or at home, economical to buy and run, the Flowtron Intermittent Compression System is well worth investigating. Ring or write for details and free copy of 'Physiology of Stasis.'

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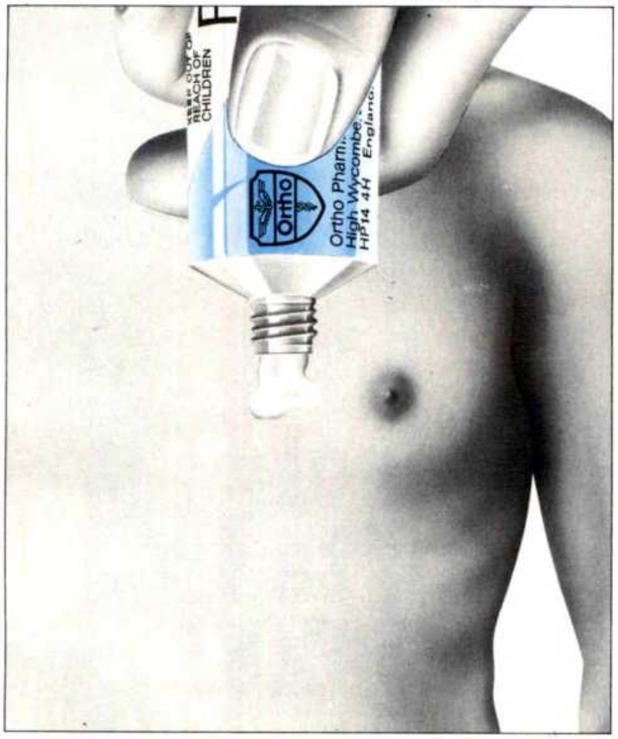
Cream **Econazole Nitrate 1%**



For All Skin Mycoses

You can take immediate action against tinea corporis and other mycotic skin infections with Pevaryl* Cream... because Pevaryl* Cream has been proven active against every type of fungal skin pathogen.[1]

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1. Thienpont, D. *et al* (1975) Arzneim. Forsch. **25,** 224-230. 2. Raab, W. (1977) Curr. Ther. Res. **22,** 65-82. 3. Dorn, M. *et al* (1975) Munch. Med. Wochenschr. **117,** 687-692.

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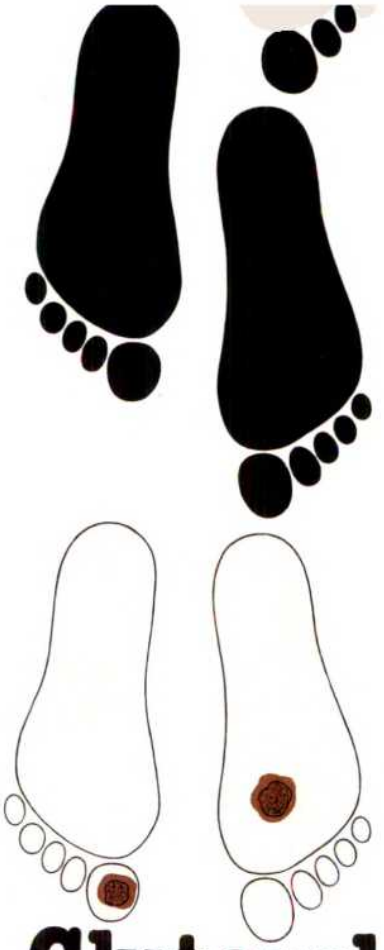

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Clinical and Laboratory Investigations

Perioral dermatitis: a 12-year review

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SUMMARY

A continuing study of perioral dermatitis over a period of 12 years is presented. A distinction is made between various patterns of circumoral and paranasal dermatitis and the clinical picture that we describe as 'perioral dermatitis'.

No cases were seen before 1966 but the number of patients presenting to hospital clinics or in private practice rose dramatically until 1970–1972, after which there has been a progressive fall in numbers. In this period, 259 patients were diagnosed as having the condition and of these 203, resident in an area of some 275,000 population and seen by one or other of us on at least two occasions, form the basis of this study. There were 173 females, fifteen males and fifteen children under 12 years of age. In many cases it was possible to suggest the primary lesion for which patients sought treatment. In sixty-nine patients this consisted of a para-oral eruption on the side of the chin and, in forty-one a paranasal erythematous dermatitis. The characteristic appearance and spread of the eruption to the glabella and eyelids are described. A few patients, mostly male, showed lesions around the eyelids only, from treatment of seborrhoeic dermatitis. All but nine of the patients acknowledged the use of potent (not necessarily fluorinated) topical corticosteroids over long periods; in many cases these were self-administered. Some of the children had been treated with preparations intended for their mothers.

The numerous aetiological agents that have been incriminated as causes of this eruption are examined. We believe that the prolonged use of topical potent corticosteroids is the only one tenable from our observations. The role of hormonal factors and of local irritant agents is examined in this context.

Tetracyclines are curative in the great majority of patients in 6 weeks if local corticosteroids are discarded. Relapses are rare. Perioral dermatitis is a most satisfying condition to treat but several questions remain unanswered. The current 'revolt' against all forms of cortisone may enable us to answer some of these in the near future.

In 1968 Hjorth *et al.* described forty cases of perioral dermatitis seen in Denmark and England during the previous 18 months. We concluded our paper with the observation that 'epidemiological studies on regional variations in the incidence of perioral dermatitis would seem to offer the best hope of obtaining a clue as to the factor or factors which induce it'. At that time these were unknown or entirely

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speculative but subsequently, several studies, notably those of Sneddon (1972) and Weber (1972), have demonstrated the role of strong topical corticosteroid preparations in the aetiology of this condition. Nevertheless, this has not been entirely accepted outside Great Britain and recent papers have considered many other aetiological factors. The opportunity afforded by a study of this distinctive disease over a period of 12 years in one area of South-East England has allowed us to examine some of these factors and to offer an explanation for the progressive rise and subsequent fall in the number of cases seen during this period.

DIAGNOSIS

Since the term 'perioral dermatitis' (Mihan & Ayres, 1964) is not an exact one and is open to different interpretations, we should make clear to what we are referring. The term conveys to us a very characteristic circumoral eruption of which, as Sneddon (1972) rightly says, 'the diagnosis is simple because its features are so distinctive'. Marks & Black (1971) have defined it as a 'persistent erythematous eruption composed of tiny papules and papulopustules distributed around the mouth'. It has been abundantly illustrated in many previous publications (e.g. Hjorth *et al.*, 1968; Sneddon, 1972). Perioral dermatitis has been described in the U.S.A. and Europe under several titles that associated it with rosacea or the seborrhoeic state. We believe this to be misleading and would wish particularly to distinguish it from the more localized papular or papulosquamous eruption affecting the side of the chin of women in the third or fourth decades and from the 'paranasal' dermatitis seen in younger women. Both of these may, as we shall show, become converted into a true perioral dermatitis on 'therapeutic' provocation; but they do not, alone, constitute the disease. Nor do they spread to involve the glabella and eyelids as does perioral dermatitis.

The nature of the initial eruption for which the patient sought treatment is not always obvious and one has to rely on the history. This is often unclear or clouded by the lapse of time. It was, however, possible to make a reasonable assumption in most cases. Whatever the initial site of the lesion, the subsequent course is similar in all cases. Over the subsequent weeks or months erythema, scaling and grouped micropustules extend around the whole of the perioral area, characteristically sparing a narrow zone close to the vermilion margin of the lips. The relative frequency of the individual lesions varies from person to person and is somewhat different in children. But the overall effect is remarkably consistent and the diagnosis can usually be made with some confidence as soon as the patient enters the room. The cheeks are spared, though some extension may occur outwards from the sides of the nose. A more characteristic extension occurs to the glabella and to the eyelids, though not to the eyebrows. Flushing may be present in some older women but it not a common feature and is not as labile as in the rosaceous patient. The conjunctivae are never suffused.

Other characteristics of the established case include a marked intolerance to local applications, including cosmetics and even hot water. Intolerance to sunlight also occurs but was not as prominent a feature as in the series of Frumess & Lewis (1957). Attempts to discontinue the use of potent topical corticosteroids are followed by a rebound phenomenon similar to that seen in steroid-treated rosacea (Sneddon, 1969) and inevitably leading to their re-introduction or the substitution of even stronger preparations.

MATERIAL AND METHODS

In the 12-year period 1966 to 1977 inclusive, 259 patients were indexed as having perioral dermatitis. Of these, 203 were resident in the area and were seen on two or more occasions by one or other of the authors. These form the basis of this report. They were seen in hospital or private practice serving a

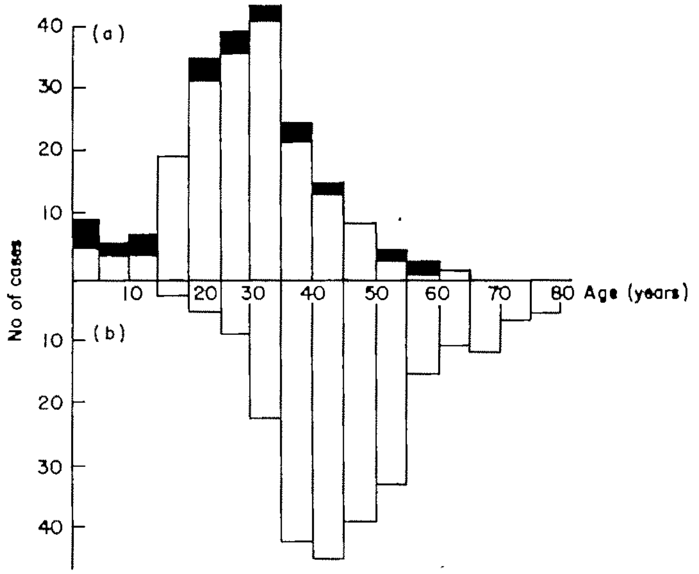

FIGURE 1. Ages of patients at presentation with perioral dermatitis and rosacea. (a) Perioral dermatitis; (b) rosacea, females only. (Open columns) females; (solid columns) males.

population of some 275,000 at the mid-term of this period. The eruption on these patients finally accepted conformed to the criteria already set out with the exception of five patients in whom a 'periorbital variation' was present (Smith, Powell & Graham, 1976). Most of the patients were seen several times and at eventual discharge were given an open appointment to enable them to return direct to any clinic in case of relapse. Few did so and subsequent follow-up letters showed that such relapse was indeed rare.

Bacteriological studies, involving a careful circumoral and nasal swab, were carried out initially on thirty-three patients and on thirty-five control patients of equivalent age and sex. Subsequently, a further twenty-three swabs were performed on patients with the disease. Patch tests were carried out on nineteen patients in 1967–1969. These comprised the Standard European Battery of the time with additional tests to cosmetics, dyes and preservatives. In 1976, a few patients were tested for immediate and delayed reactions to 0·1% and 0·4% stannous chloride.

There were 173 females, fifteen males and fifteen children under 12 years of age, three of whom were aged 10–12 years. The ages of the patients at the time of presentation are shown in the upper half of Fig. 1. The mean age of all adult patients was 30·2 years and of the adult females 29·6 years. Sixty percent of patients were aged between 16 and 35 and only seven patients were over 50 years of age.

THE ERUPTION

The patients were almost invariably referred because of a persistent perioral rash (Fig. 2) which had resisted treatment with corticosteroids; there were frequent references in the referral letter to the flare that had occurred on attempts to discontinue their use.

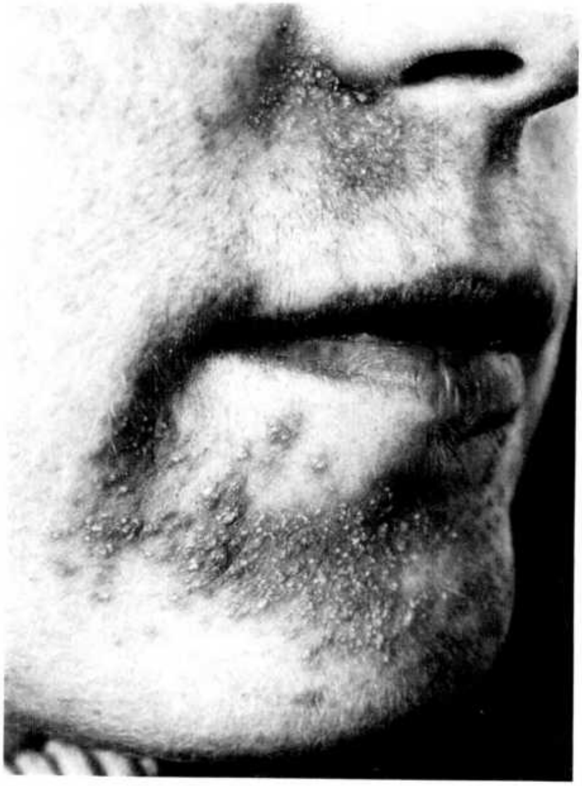

FIGURE 2. Perioral dermatitis showing erythema, grouped papular pustules and sparing of the region close to the lip.

Duration. In 178 cases in which this was recorded, the condition had been present for more than 3 years in sixteen patients, more than 1 year in sixty-four and for 6 months or less in ninety-eight. This long duration before referral for a condition that was inevitably distressing for young women reflected the temporary suppression brought about by potent corticosteroid therapy. It was the anxiety engendered by their prolonged use rather than their failure to contain the eruption that prompted many of the referrals.

Site and nature of initial lesions. Most patients were able to recall the site of the initial lesions for which they sought treatment. These are shown in Table 1. They were less well able to describe the exact nature of the lesion, though they could usually distinguish a scaly erythema from papules and pustules. Many, of course, recognized acne as such and others were well able to describe the scaly erythema of the sides of the nostrils that we propose to refer to by the term 'paranasal dermatitis'. Most mothers recognize 'dribble' eczema or dry patches of chapping around the mouths of their young children and noted that the eruption that developed subsequently was different in character. A history of pre-existing seborrhoeic dermatitis was obtained in only ten patients: four of them were men. In assessing the original condition we were therefore obliged to accept the patient's own assessment but we were able in several cases to supplement this by observing the re-emergence of the original

TABLE 1. Site of initial lesions in perioral dermatitis

Site	No.
Side of chin	69
Alae nasi	41
Side of mouth	22
Side of chin and mouth	13
Alae nasi and chin	9
Glabella or periocular	9
Other combinations	14
Unknown or uncertain	26

TABLE 2. Probable nature of original skin disease

Original skin disease	No.
Para-oral dermatitis (chin only)	38
Paranasal erythematous dermatitis	36
Acne	19
Seborrhoeic dermatitis	10
Chronic superficial dermatitis (pityriasis alba)	7
Rosacea	7
Psoriasis	5
'Dribble' or 'lick' eczema	5
Dry or flaky skin (females)	4
Other (?herpes simplex, angular cheilitis, etc.)	4

condition after cure of the perioral dermatitis. Those cases in which we felt reasonably confident about the pre-existing condition are shown in Table 2. We have deliberately retained the terms 'para-oral' and 'paranasal' dermatitis at this stage without reference to any particular aetiological connotation. The first may, in many cases, be a localized acneiform eruption of the chin affecting women of the third and fourth decade but who seldom have a preceding history of acne: the second may be a variant of seborrhoeic dermatitis. Both conditions require further study. The mean age of those with 'chin acne' was 39·6 years and that of those with paranasal dermatitis was 27·3 years, though the onset of the condition was often in adolescence.

In five instances in which the eruption was localized to the glabella and eyelids, the appearance was identical to that occurring in the extension of true perioral dermatitis and we regard it as justifiable to include this as a variant, although we recognize that they might equally well be included in other descriptions of adverse reactions to corticosteroid therapy. Most of the patients were men who had been treating seborrhoeic dermatitis of the scalp or eyebrows with potent corticosteroids. One man had been using clobetasol propionate for alopecia areata and presented with an early perioral dermatitis that had originally involved the periocular area alone.

Children. Fifteen children under 12 years of age have been included in our study. Ten were female and five male. They all had a persistent perioral eruption which had the same general features as that seen in adults but it was less florid and papulopustules were more evident than microvesicles and scaling. The eyelids or the glabella were involved in four patients and this was a cogent reason for including the others, who had a similar though atypical perioral eruption. We excluded the common cases of uncomplicated lick and 'dribble' eczema, chapping and chronic superficial dermatitis and those with the 'rosacea-like dermatitis' described by Savin, Alexander & Marks (1972). Six of the patients were 2 years old or younger; four were aged 10–12 years. Only four children were atopic but there was a family history of atopy in another two.

We had to rely on mother's description of the original rash for which topical steroids had been prescribed. In the younger children, this was usually a 'dribble' eczema or areas of chapping. In the older children, early acne or chronic superficial dermatitis were the most common. One young atopic child was said to develop a perioral rash after eating grapefruit and immediate-type reactions were evoked on skin-testing with citrus fruits. It was notable that five of the children were offspring of mothers who themselves had been given fluorinated corticosteroids for their perioral lesions. Other mothers had used these preparations on their own initiative from the family store of medicaments and without the knowledge of their doctor, for these rashes. They were seen, therefore, as a result of direct questioning rather than as a result of formal referral.

Unusual features. Apart from the 'periocular' variety that has been referred to already, two patients presented with a purely unilateral eruption, in one case even involving only the right side of the glabella. The patients did not associate this with habitually sleeping on that side.

Another rather unusual feature that has been noted by all of us was the frequency with which medical or paramedical families were represented. Four patients were doctors' wives and five were nurses or physiotherapists. It might be cynical to suggest that they had easier access to topical corticosteroids than most.

Spread of the eruption. Though patients were often unable to specify the exact sequence of spread of the eruption, it was often possible to determine the nature of the spread beyond the perioral area. This is summarized in Table 3. This extension to the glabella and the eyelids appeared to be more common among those whose original lesions were at the sides of the nostrils, suggesting a susceptibility for the whole central area of the face to become involved in these patients. Spread from the periocular area to the perioral region occurred in three males.

The rise and fall of perioral dermatitis. The most striking feature of this 12-year series is the rapid increase in the number of cases seen each year, from the first two recognized cases late in 1966 to a peak

TABLE 3. Pattern of spread beyond perioral area

Pattern of spread	No.
To glabella only	
from alae nasi	9
from other initial areas	15
To glabella and eyelids	
from alae nasi	5
from other initial areas	6
To eyelids alone	11
From glabella and eyelids to perioral area	3

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TABLE 4. The rise and fall of perioral dermatitis

Years	Cases
1966–68	25
1969–71	85
1972–74	59
1975–77	34

of thirty-six cases in 1970. The number remained high until 1973, after which they progressively declined to thirteen in 1976 and twelve in 1977. No cases were recognized before the end of 1966. These figures are summarized in Table 4 and have been illustrated elsewhere (Wilkinson, 1977) on a larger number of cases based on the total number of indexed cases up to 1976. We believe that this recent clear and sustained fall in the annual number of cases seen is important in considering the aetiology.

INVESTIGATIONS

In the early days of recognition of this apparently new pattern of dermatitis, the cause was entirely unknown and we were not convinced by the explanations that had so far been suggested. The questions that we asked our patients at this time and the investigations we set in motion proved unrewarding as the numbers of patients increased and there is no point in detailing them.

Bacteriology. Of the seventy-six circumoral swabs taken, twenty-eight grew no organism. Coagulase-negative staphylococci alone or with other organisms were grown from twenty-eight patients and diphtheroids from eleven. Coliform organisms were cultured seven times. The figures were little different in proportion to those obtained from control subjects, except that a larger number of controls (18/35) grew no organism.

Patch tests. Only five positive results were obtained from the nineteen patients tested. Three were irrelevant—nickel, neomycin and colophony. One positive reaction to lanolin and one to Balsam of Peru might have been related to cosmetic usage but after resolution of their eruption these and other patients were able to resume the use of their usual make-up without ill effect. The patients' own cosmetics, tested as such, were negative.

Histopathology. We felt reluctant to carry out routine biopsies on the faces of young women who were already distressed by their appearance. We did so on only four patients, all typical and rather severe. One showed a follicular pustule, with no organisms on Gram stain and two showed mild perifollicular inflammatory changes and a perivascular round cell infiltrate, similar to the changes noted by Marks & Black (1971). The fourth, from the chin of a woman of 46 years, showed a granulomatous reaction, as described by Mullanax & Kierland (1970) in their one case of presumed perioral dermatitis. Our patient was one of the seven in whom rosacea may have been the original reason for topical corticosteroid therapy. We looked for but found no evidence of *Demodex folliculorum*, as suggested by Bendl (1976).

Hormonal factors. Sixteen patients developed the eruption while pregnant, but at no particular period of cyesis. The proportion is no greater than would be expected among 179 females, mostly of child-bearing age. Five patients were affected in the first 3 months after delivery; in one case the first lesions appeared soon after an abortion.

Of 117 women questioned, forty-five were taking the contraceptive pill, sixty-eight were not and four had recently discontinued it. Of those whose initial lesions were on the chin, twenty-three were taking the pill and thirty-four were not. Only seven patients stated that the eruption had begun within 3 months of starting this form of contraception. No other correlation was found often enough to be significant.

Fluorinated toothpaste. In view of the suggestion by Melette, Aeling & Nuss (1976) that fluoride in toothpaste might be a cause of the papular and vesicular lesions of perioral dermatitis, a questionnaire was sent to thirty-seven patients treated and discharged from 1974–1976 and to fifteen patients seen before 1974. Replies were received from thirty in the first group and seven in the second. Twelve patients continued to use a fluorinated toothpaste of whom ten remained cured and two had slight recurrences of their original condition (but not true perioral dermatitis). Eighteen had not used these toothpastes although twelve had begun to do so since discharge. Fifteen remained cured and three had some recurrences of their original condition. Seven patients did not know what type they used or changed brands frequently.

Use of topical corticosteroids. All but nine of our patients gave a clear history of the use of topical corticosteroids, though in some cases this was given reluctantly or only on the second visit. It was evident that some patients had developed feelings of guilt about their use. This was rendered more acute by their failure to accept the rebound that followed attempts to leave off their use. In many cases the preparations had been used without the knowledge of their doctor, being obtained from relatives and friends or from the household medicament chest. In 136 cases the corticosteroid was either betamethasone 17-valerate or fluocinolone acetonide; three patients had used clobetasol propionate. In thirteen cases other topical corticosteroids of the potent group had been used. In three of these beclomethasone dipropionate alone was named. In five patients it was only possible to elicit a history of the use of weaker preparations such as fluorandrenolone (3) or of hydrocortisone itself (2).

One of the nine patients who denied the use of all topical corticosteroids was a doctor's wife who developed a very typical perioral dermatitis in pregnancy and in whom this continued until effective treatment could be given after the delivery. Nevertheless, the reluctance of some patients to admit to the use of these agents, especially when not prescribed by their doctor, led us to wonder whether recollection of their use might be unconsciously suppressed. However, we have no evidence to support this.

TREATMENT

In the early period of the recognition of perioral dermatitis attempts at treatment were mostly based on local therapy. This was usually badly tolerated and it was evident that the affected skin had a very marked intolerance to agents normally used in this area without ill-effect, e.g. ichthyol, sulphur and even bland creams. Hydrocortisone cream and ointment were better tolerated but relatively ineffective, except in some children after withdrawal of stronger corticosteroids. Antibacterial preparations, including chlortetracycline in propylene glycol, were either ineffective or caused irritation. Metronidazole by mouth was given to two patients without effect but co-trimoxazole appeared to be helpful in two of three cases in children.

Towards the end of 1968 one of us (V.K.) began to use oral tetracyclines on an empirical basis. Oxytetracycline, being the cheapest, was used and we have found no reason to use any other subsequently. It was given as for acne, 250 mg twice daily, at least 3/4 h before or 2 h after a meal. The response was dramatic, provided that all topical treatment was suspended. The particular importance of discontinuing the use of topical corticosteroids gradually became evident from the indifferent response or early relapse of these who did not do so. The distinction was so sharp that it left little doubt as to the importance of these agents, at least in protracting the condition. One patient did not respond to tetracyclines until she had left off using topical hydrocortisone. By the end of 1969 a satisfactory standard therapeutic regime had been established which was based on a dose of 250 mg tetracycline twice daily for 3 weeks and then once daily for a similar period. It was later found possible to reduce the initial period in mild cases to 2 weeks. Children and women who were pregnant, who were trying to become pregnant or who were breast feeding were not so treated. In most cases the condition continued or resolved only slowly with the use of hydrocortisone topically; latterly, hydrocortisone butyrate has been helpful in such cases, as Sneddon has found (Sneddon, 1973), particularly in controlling the rebound period. We have not yet seen any case of perioral dermatitis develop from its use but it was not being prescribed widely during this period.

In all, 116 patients were treated with oxytetracycline. Five patients needed two courses and three patients required three. But the response to a single course was usually perfectly satisfactory. Eighty of these 116 patients were seen again after the end of the course of treatment, sixty-two at an interval of 1–3 months, nine at 6–12 months and nine from 2–5 years later (when they attended for other conditions). Five patients relapsed; two admitted using strong topical corticosteroids again—usually after interval of time during which they thought they might have been immune to their effects.

In a letter sent in 1976 to fifty-two patients treated in the previous 5 years and not still attending hospital, patients were asked whether they were free of the eruption. Thirty-seven patients answered. Twenty-eight had remained perfectly clear and nine had had recurrences of their original condition—paranasal dermatitis, seborrhoeic dermatitis, etc. None had had a recurrence of perioral dermatitis as we define it and only one had used a topical corticosteroid—hydrocortisone.

COMMENTS

The mean age of the patients in this series and the high proportion of females accord closely with those of other reported series. An explanation of the larger number of children included has been advanced. These presented with lesions similar to those described by Savin *et al.* (1972), Thune (1974) and others. The mothers themselves were sometimes suffering from the same condition, as has been noted by Sneddon (1972) and Verbov & Abell (1968).

The relationship to rosacea. Early accounts of perioral dermatitis emphasized the role of light sensitivity (Frumess & Lewis, 1957) or of seborrhoea (Kaufman, 1965). In subsequent European publications, it has often been referred to as 'rosaceiform', 'rosacea-like' (Baran & Civatte, 1971; Petzoldt, 1971; Weber, 1972). One of us (Wilkinson, 1977) has presented elsewhere the evidence against considering perioral dermatitis to be a form of rosacea. To start with, the distribution of the lesions is different. The cheeks are rarely involved, the nose never. The open V-shaped flush of the forehead is absent and the characteristic and easily evoked conjunctival suffusion of the rosaceous patient (Starr & Macdonald, 1969) does not occur. On the other hand, the sparse, isolated, papular, periorbital lesions of perioral dermatitis are not a feature of rosacea.

A comparison of the ages at presentation of patients with rosacea and with perioral dermatitis is shown in Fig. 1. These patients were all seen in the same area by the same observers during the same period. The mean age of 179 female patients with perioral dermatitis was 31·2 years, over half being between 20 and 40 years old. The mean age of the female patients with rosacea was 44·3 years, half presenting between the ages of 35 and 50. A comparison of the proportion of patients presenting between 15 and 35 years shows an even more striking difference—58% in perioral dermatitis and only 17·5% in rosacea. There is also a great disparity in the sex rate. Only fifteen of 198 patients with perioral dermatitis were adult males. During the same period, sixty-seven males were seen with rosacea. The proportion of male and female patients with this disease was similar before and after 1966, irrespective of the emergence of perioral dermatitis. Moreover, the numbers of patients seen with rosacea fluctuated from twenty to thirty-seven annually throughout the period in which perioral dermatitis showed its marked rise and decline.

Finally, Marks & Black (1971) have demonstrated the histological distinction between the two diseases in their substantial series of biopsies. The authors have seldom had any difficulty in distinguishing between the two diseases and are convinced that they should be regarded as separate entities, even though occasional cases of rosacea (six in this series) may develop perioral dermatitis under the influence of topical treatment.

Other differential diagnoses. Patients with acnitis show a more diffuse papular eruption and healing is slow, uninfluenced by tetracyclines and followed by scarring. The rosaceiform trichophyton infection described by Grupper, Cardinne & Avram (1975) has its own distinctive characteristics. We did not find that many of our patients were markedly seborrhoeic; some complained of having dry skins. Seborrhoeic dermatitis (other than paranasal dermatitis) was the probable initial complaint in only ten of our patients (Table 2) and we agree with Macdonald & Feiwel (1972) that perioral dermatitis should be distinguished from this disease.

Aetiology. Despite the clear demonstration by Sneddon (1972) of the association of perioral dermatitis with the topical use of potent corticosteroids and the general acceptance of this view in Great Britain, other causes of the eruption have continued to be proposed. It is not always clear, however, whether authors are referring to the same condition as we describe. Gschwandtner (1974) has suggested that a light-sensitive perioral eruption may be a separate entity. Certainly light sensitivity was not a notable feature of our cases.

The role of local applications and of cosmetics is difficult to assess. In his large Russian series of 329 patients, Arujonow (1978) listed a wide variety of substances he believed to have acted as irritants and Bendl (1976) was particularly suspicious of moisturizers. We did not find, as he did, any significant difference in the cosmetics used by our patients. Many of the younger ones had never used cosmetics and others had stopped using them without amelioration. Undoubtedly, patients with perioral dermatitis become intolerant to substances normally well tolerated. We regard this heightened irritancy, which was noted by Kaufman (1965) as similar to that occurring to dithranol on psoriatic patients who have been treated with potent topical corticosteroids.

Infectious agents of varying types have been implicated by European authors, especially *Candida albicans* (Bradford & Moutes, 1972) and fusobacteria (Schubert & Roc̆kl, 1972; Kalkolf & Buck, 1977). We would agree with the latter authors that topical corticosteroids may encourage the emergence of such organisms. Our findings suggested that bacterial infection was more prevalent in our cases than in control patients, but no one species was predominant. We searched for corynebacteria on several occasions but we were unable to show their presence in excess. However, the response to tetracyclines suggests that infection may play an important secondary role in the disease.

In any disease of obscure origin affecting the female sex, it is inevitable that either hormonal or psychosomatic influences will be adduced as a cause. Wilsch & Hornstein (1976) studied 279 patients, two-thirds of whom had been treated with topical corticosteroids, and considered that 90% were suffering from psychosomatic or 'neurovegetative' disorders and that the patients showed a marked 'zeal to advance'. Our patients were drawn in equal proportion from a small industrial and rural community and from private practice in a commuter belt population. Though psychological studies were not carried out, none seemed to us to show more general distress than would any young woman suffering from a central facial eruption lasting for months or years. Nor were we impressed by the role of cyclical hormonal changes, pregnancy or the contraceptive pill and we agree with Macdonald & Feiwel (1970), Sneddon (1972), Weber (1972) and other authors in this respect. Some cases started in pregnancy, some after pregnancy; most had no relation to this or to the patient's menstrual history. In particular, the number of women taking the contraceptive pill increased steadily from 1965 to 1975, with a marked rise from 1971–1974, when our cases were declining in number.

We were entirely unable to substantiate the supposed role of fluorinated toothpastes (Mellette *et al.*, 1976). During the period in which the number of our cases increased and then fell progressively, the share of the U.K. market carried by such toothpastes rose from 8% in 1968 to an estimated 85% in 1976 (Banks, personal communication).

Topical corticosteroids. There remains the role of potent (not necessarily fluorinated) topical corticosteroids. We have no doubt that the misuse of these highly effective and very useful preparations offers the most satisfactory explanation of the pattern and behaviour of perioral dermatitis in our series and confirms the findings of Sneddon (1972) and those of Weber (1972) and, more recently, of Nortung & Parsmann (1977) and others. We offer the progressive and subsequent fall in numbers seen annually as evidence for this. The potent topical corticosteroids were introduced about 1960 and increasing numbers of reports of their use followed in the next few years. Early papers emphasized their value (Robinson, 1961; Samman & Beer, 1962). There is normally a gap of 4 or 5 years before a new product is widely used by general practitioners. In our area, these were being extensively prescribed from about 1965 onwards. During this period dermatologists were not exempt from the habit and prescribed these more widely than they would do nowadays. Most of the patients we have described have been using betamethasone 17-valerate. This was a matter of local preference stemming, no doubt, from the involvement of one of us in the initial trial of this agent (Williams *et al.*, 1964). Several other corticosteroids were also used. Among these were two non-fluorinated preparations. This caused us to hesitate in accepting the concept advanced by Fisher (1975) of a contact halogenosis. Only two patients admitted to the prolonged use of hydrocortisone itself; but our satisfactory use of this corticosteroid in treatment leads us to suspect the validity of this. We have noted the frequency of self-medication in the prolonged period during which these corticosteroids were used. In nine patients, however, we could obtain no history of use of these agents. Sneddon (1972) had a similar experience. We feel that this does not detract from the tenor of our argument that these agents were responsible for the eruption in almost all—if not all—of the patients we saw.

The increasing recognition of the dangers of use of these agents on regions such as the face was transmitted in our area to the general practitioners by means of repeated post-graduate talks and through a circulated newsletter. In addition, the message was beginning to be delivered in the general and more popular medical journals and news-sheets. We believe that the progressive fall in the number of cases since 1971 is attributable to the dissemination of this knowledge and an increasing reluctance of the general practitioner to prescribe them. The message, however, did not reach the level of the consumer direct until the early part of 1978, when drugs in general and steroid preparations in particular were given a bad press. It will be interesting to see how this is reflected in future figures.

Treatment. Several authors independently found tetracyclines of value at about the same time

(Sneddon, 1972; Macdonald & Feiwel, 1972; Thune, 1972), provided that the use of corticosteroid was prohibited at the same time. Failure to do this may explain some of the poor results quoted by others or the prolonged courses that were found necessary. As early as 1971, Miller (1971) found that a 6–8 week course of tetracycline was satisfactory. Withdrawal rebounds were originally controlled (rather poorly) by hydrocortisone but almost completely by hydrocortisone 17-butyrate (Sneddon, 1973).

We have found the control of the eruption more difficult in young children and particularly in young women thought to be, or desirous of being pregnant. Metronidazole has been given in three recent cases but without marked success. Topical therapy is poorly tolerated and has to be extremely mild. We have found hydrocortisone 17-butyrate to be of value in the initial stages, after which topical therapy has appeared to us to be tolerated rather more easily. But the numbers are too few for further comment.

True relapses have been rare and some at least were occasioned by the re-introduction of topical steroid therapy. Rather more common has been the re-emergence of the lesions which required treatment in the first place but even this has been comparatively uncommon or when it has occurred, mild enough not to worry the patient particularly.

CONCLUSION

In incriminating the role of strong corticosteroids in perioral dermatitis, a provisional hypothesis of the sequence of events might be as follows: the patient presents with one of the original conditions mentioned and treatment with strong topical corticosteroids is either prescribed or self-administered. Attempts to leave this off are followed by the return of the condition and the resumption of treatment. The effect of this is to cause thinning and erythema as is seen in rosacea (Sneddon, 1969) and also a diminished tolerance to irritancy from common contactants—cosmetics, therapeutic and environmental. They may also induce the emergence of organisms of one type or another, perhaps depending upon racial or cultural differences. Although we do not support the view that hormonal factors play a major part, it remains an intriguing problem as to why the central facial area is so selectively affected. With the increasing aversion of patients themselves to use any form of topical corticosteroids, the answer to some of the questions may emerge in the next few years.

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Perioral dermatitis

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SUMMARY

Forty-three patients with perioral dermatitis are described. In seven patients, this eruption was associated with the use of hydrocortisone butyrate (Locoid) alone, and this topical steroid can no longer be recommended unreservedly for use on the face. In fifteen other patients, medication was undertaken without medical advice. The time may have come to print on tubes of potent local steroids warnings about the hazards of long-term use on the face without medical advice. Drug companies and pharmacists could also help by asking patients to discard any partially-used tubes of ointment and an expiry date stamped prominently on the tubes rather than on the cardboard container may help. Continuing medical education is obviously still required to prevent a minority of family doctors prescribing wrongly for their patients.

Perioral dermatitis is a relatively new dermatological disorder, possibly first recognized by Frumess & Lewis (1957). Later cases were reported by Mihan & Ayres (1964), Hjørth *et al.* (1968) and Finley (1969). The pathogenesis of this condition remained unknown until Sneddon (1972) incriminated the use of topical fluorinated steroids on the face. In addition, Sneddon noted that the topical steroids used by the patient need not have been prescribed by a doctor, but were often used on the recommendations of friends or relatives and illustrated this fact by describing three affected members of a pharmacist's family with ready access to topical fluorinated steroids.

Perioral dermatitis continues to be a fairly common clinical problem and it was the author's impression that the proportion of patients prescribing fluorinated steroids for themselves is increasing. Furthermore, several patients have been seen recently with perioral dermatitis associated with the use of hydrocortisone butyrate, whilst Sneddon (1973) had advocated this local steroid preparation for the treatment of perioral dermatitis.

It was decided, therefore, to examine in detail the case records of forty-three patients with perioral dermatitis seen predominantly in private practice in the Leeds and Harrogate area since 1974.

PATIENTS

Forty-three patients, forty-one female and two male (age range 16–57 years, mean 32·2 years), were studied from January 1974 to April 1978. Twenty-one of the forty-three patients had a clear past

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medical history of skin disease, the majority having atopic or seborrhoeic eczema, acne or pompholyx. All the patients had used local steroids, these local steroids being prescribed originally, and up to 12 years previously, by the patient's family practitioner (thirty-three patients), consultant dermatologist (three patients), patient's relatives, or pharmacist (fifteen patients).

In the fifteen patients who treated themselves without consulting their doctor, the sources of the local steroid were many and varied. The commonest source was a supply of fluorinated steroid kept at home after an episode of probable eczema in the family years before. A local pharmacist supplied a fluorinated steroid without prescription in one case; a pharmacist husband supplied the medication to his wife in two cases; 'Mum' supplied the ointment in two cases; one patient used her son's Betnovate ointment, and one patient used a steroid available in the sick bay at work, one patient obtained a supply in Saudi Arabia, and one patient found a tube in a house she had bought and used it.

The steroids used are shown in Table 1, the majority of affected individuals having used betamethasone valerate (Betnovate) 74%, fluocinolone acetonide (Synalar) 28%, hydrocortisone butyrate (Locoid) 16%, clobetasol propionate (Dermovate), flurandrenolone (Haelan), and clobetasone butyrate (Eumovate). Twenty-three of the forty-three patients had used only one local steroid, whilst thirteen patients had used two, five patients had used three different preparations, and two patients had used four different local steroids.

TABLE 1. Various steroids used by the patients

Steroids used		Patients
Betamethasone valerate	(Betnovate)	32 (74%)
Fluocinolone acetonide	(Synalar)	12
Hydrocortisone butyrate	(Locoid)	7 (16%)
Flurandrenalone	(Haelan)	
Clobetasol proprionate	(Dermovate)	4
Clobetasone butyrate	(Eumovate)	3
Triamcinalone acetonide	(Adcortyl)	
	(Triadcortyl)	2
Fluclorolone acetonide	(Topilar)	
Fluocortolone pivalate	(Ultradil)	1
Fluocortolone hexanolate	(Ultralanum)	1
Beclomethasone dipropionate	(Propaderm)	1
Fluocinonide	(Metosyn)	1
Neomycin/methylprednisolone	(Neomedrone)	1

Ten of the female patients had been under severe emotional stress before presentation, and one patient had frank anorexia nervosa. Four of the female patients were taking the contraceptive pill, and in two of these patients acne had been the initial dermatological problem.

All patients were treated with oral tetracycline in a dose of 250 mg either once or twice daily and the potent local steroid was withdrawn and either 1% hydrocortisone ointment or Alphaderm ointment substituted. All patients were better in 3 months and most had normal skin after 6 weeks of this treatment.

DISCUSSION

The patients described in this series are very similar to those described by other authors in that they are predominantly female with a mean age of 32·2 years (Hjørth *et al.*, 1968; Finley, 1969; Sneddon, 1972; Sneddon, 1973). Moreover, all the patients had been treated with local corticosteroid ointments and creams and all recovered when these agents were withdrawn. Thus, there can be little doubt that potent local corticosteroid preparations are important in the pathogenesis of this condition. However, other factors in pathogenesis must also be considered. Almost half the patients had a history of previous skin disease and atopic eczema was common in the self-medicating families. A quarter of the patients had also experienced a period of recent severe emotional stress. It is also interesting that four of the patients had presented 1–4 months after taking the contraceptive pill. Two of these patients had initially developed acne and had been treated inappropriately with a local corticosteroid. The androgenic progestins contained in many contraceptive pill formulations may stimulate facial sebaceous glands and thus permit greater 'shunting' of active corticosteroid into the skin.

It is known that self-prescription for skin disease is much more common than doctor prescription (Office of Health Economics, 1973). Moreover, apart from analgesics, skin preparations are found in a higher proportion of homes than any other type of medication (Dunnell & Cartwright, 1972). It is not surprising, therefore, that members of the general public will self-medicate with local steroids, and whilst the sources of supply were very variable, one of the commonest was ointment retained by households after initial use years before the eczema.

Whilst the majority of affected individuals had used fluorinated steroids, seven patients had used hydrocortisone butyrate (Locoid) alone. The hydrocortisone butyrate (Locoid) had been prescribed without exception by medical practitioners, and in one case by a consultant dermatologist. Locoid has been advocated in the management of steroid-induced rosacea and perioral dermatitis (Sneddon, 1973). However, in eight cases of perioral dermatitis treated by Sneddon (1973), six also received oral tetracycline in a dose of 250 mg twice daily. Whilst hydrocortisone butyrate may be effective in perioral dermatitis, when combined with oral tetracycline, it also seems capable of inducing perioral dermatitis in some patients when used on its own. The author can no longer recommend the routine long-term use of hydrocortisone butyrate (Locoid) on the face.

It is not surprising that Locoid can induce perioral dermatitis on the face, because it has been shown that hydrocortisone butyrate can induce greater thinning of human skin than betamethasone valerate (Kirkby & Munro, 1976). Moreover, hydrocortisone butyrate can produce more dermal atrophy than betamethasone valerate (Dykes & Marks, 1977). In addition, hydrocortisone butyrate and betamethasone valerate are equally potent in producing epidermal thinning and reduction in epidermal cell size in humans (Delforno, Holt & Marks, 1978). Barry & Woodford (1977) have shown that, whilst Locoid was less potent in producing a blanching response than betamethasone valerate, it was significantly more potent than Alphaderm or 1% hydrocortisone cream. These experimental data are in accord with my clinical findings.

The steroids used most commonly by patients in this series were Betnovate and Synalar and this may reflect their popularity, both with patients and with practitioners in this country. Because 35% of the cases of perioral dermatitis described in this series were due to self-medication and because self-medication is a strong cultural characteristic of western society, the writer feels that the time has now come to label tubes of fluorinated steroids with a warning about their use on the face without resort to medical advice. An expiry date clearly stamped on the tubes rather than on the cardboard containers may also be helpful.

A worrying feature of this series is that a minority of general practitioners continue to prescribe potent corticosteroid ointments for facial dermatoses, despitee quit widespread publicity. Perioral dermatitis is an eminently preventable disease.

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Studies on fibronectin in the skin

I. INDIRECT IMMUNOFLUORESCENCE STUDIES IN NORMAL HUMAN SKIN

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SUMMARY

Fibronectin is a glycoprotein mediating contact between cellular elements and collagen. As judged by indirect immunofluorescence studies fibronectin is abundantly present in normal human skin. It is located in the dermo–epidermal junction area, in the papillary and reticular dermis, about epidermal appendages (pilosebaceous units and eccrine sweat glands) and in the vascular and neural structures.

Morrison, Edsall & Miller (1948) isolated a glycoprotein from Cohn-fraction I of human plasma, called cold insoluble globulin (Cig). In the last few years, Cig has been further characterized in human plasma by Mosesson & Umfleet (1970) and from a plasma cryoprecipitate called 'heparin precipitable fraction' by Fyrand & Solum (1976). At the same time, different research groups have worked with a fibroblast surface protein which has been found to be identical with Cig from plasma, including immunological identity (Ruoslahti *et al.*, 1973; Ruoslahti & Vaheri, 1974, 1975). This fibroblast surface protein has received various names. In the present study, tissue Cig is called fibronectin, indicating its binding to fibrous proteins (Keski-Oja, Vaheri & Ruoslahti, 1976).

Fibronectin constitutes a substantial part of the connective tissue, representing an 'adhesion' factor (Klebe, 1974) and a 'spreading' factor (Grinnell, 1976) in fibroblast tissue cultures. Plasma Cig is probably an important opsonic agent demonstrating stimulation of macrophages (Blumenstock, Weber & Saba, 1977), and is a substrate for activated Factor XIII in the stabilization of fibrin (Mosher, 1975).

Fibronectin seems to be important in the adhesion of collagen to tissue elements such as fibroblasts (Klebe, 1974) and in the linking of normal cells to each other by a fibronectin-containing matrix (Chen, Gallimore & McDougall, 1976). A comprehensive review on fibronectin has recently been published by Yamada & Olden (1978).

By indirect immunofluorescence techniques fibronectin has been demonstrated in the basement membranes of lungs, kidneys and skin (Linder *et al.*, 1975). It is an important constituent in the physi-

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ology and pathophysiology of the skin. As no systematic study on this protein has been reported in normal skin, this is the aim of the present investigation.

MATERIAL AND METHODS

Biopsies were taken from normal skin from ten out-patients from the surgical department. The group consisted of six women, mean age 40 years and four men, mean age 41 years. The biopsies were quick-frozen in Tissue-Tec OCT compound (Lab-Tek Products Division, Illinois, U.S.A.) using dry ice in acetone-isopentane and stored at −25°C until used.

Antiserum against plasma cold insoluble globulin/fibronectin

Plasma Cig was isolated from pooled EDTA-plasma from dermatological in-patients with different diseases. Using affinity column chromatography (Gelatine-Sepharose) (Engvall & Ruoslahti, 1977) the plasma proteins were eluted with a starting buffer (0·05 M Tris-buffered saline containing 5 mM glucose and 0·6 mM EDTA, pH 7·4), whereas plasma Cig was selectively absorbed to the column because of its specific affinity to collagen.

With a second buffer (8 M urea in 0·05 M Tris-buffered saline, pH 7·4) plasma Cig was flushed from the column. The eluted Cig fraction was found to be free from other plasma proteins as judged by SDS-polyacrylamide gel electrophoresis, immunodiffusion, crossed immunoelectrophoresis and quantitative electroimmunoassay. Antiserum against plasma-Cig/fibronectin was raised in rabbits by multiple injections of the eluted plasma-Cig fraction into the skin of the neck yielding a good antibody response. The antiserum was made mono-specific by absorbing with the eluted plasma-Cig free plasma proteins in the starting buffer.

Indirect immunofluorescence technique

Skin samples were cut in a cryostat at 4 μm and stained using a 'sandwich technique' essentially according to Beutner, Chorzelski & Jordon (1970). As the first layer, the absorbed monospecific anti-Cig rabbit antiserum was used, diluted 1/32 with phosphate-buffered saline, pH 7·3 (PBS), containing 4% bovine serum albumin. At the second layer a FITC-conjugated goat-anti-rabbit-immunoglobulin serum was used (F/P-ratio 4·0) (Behring, W. Germany), diluted 1:10 in PBS containing 4% bovine serum albumin. The staining time of the second layer was 15 min. The specificity of the immunofluorescence (IF) was tested by blocking experiments with purified plasma Cig from heparin precipitable fraction (Fyrand & Solum, 1976), demonstrating a total loss of IF. Sections with normal rabbit sera as the first layer were used as controls. The sections were embedded in glycerol/PBS 1:1.

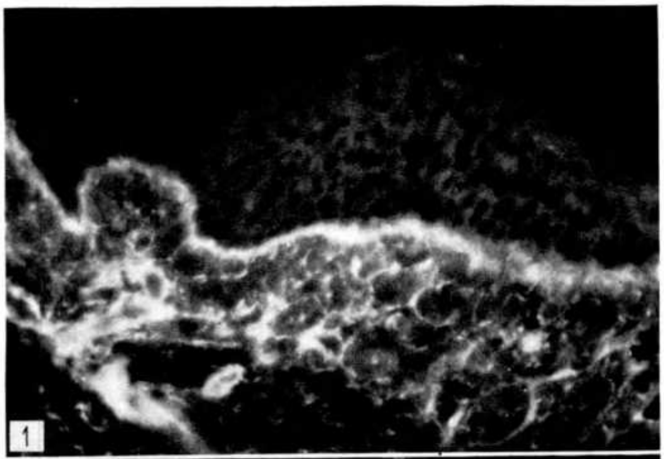
Microscopy and microphotography

A Leitz Orthoplan microscope equipped for transmitted light from an Osram HBO 50 W high pressure mercury lamp was used. Filter combinations for FITC were primary filter BG 12 and secondary filter KV 510. Microphotographs were taken through a Leitz vertical illuminator with an Orthomat camera, with Agfachrome 50L professional film, and with an exposure time of 30–45 s.

RESULTS

Epidermis. In the epidermis, no specific staining for fibrinectin was observed.

Basement membranes. Fibronectin was demonstrated as a continuous linear staining of the basement membrane (Fig. 1). The intensity of this staining was relatively homogeneous in the same person,



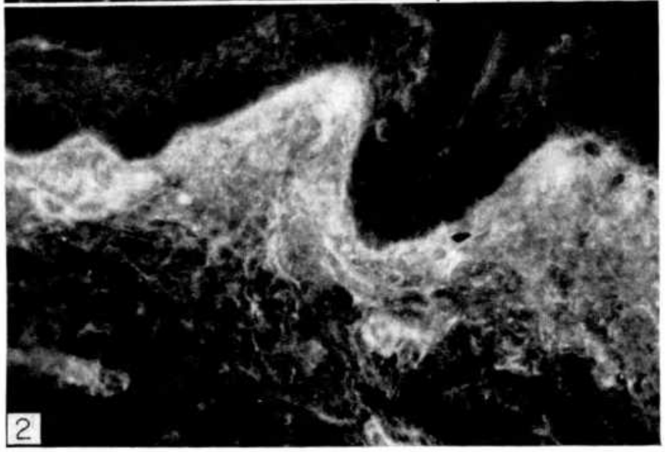


FIGURE 1. Fibronectin in a linear staining at the dermo–epidermal junction and a reticular staining of the papillary dermis.

FIGURE 2. Prominent reticular staining in the papillary dermis.

but in sections from different persons it varied from faint to very strong although always present. In most cases the staining was linear and homogeneous, but in a few sections patchy areas could also be observed.

Papillary dermis. In all samples, a reticular pattern of IF could be demonstrated in the papillary bodies and in the subpapillary region. In most cases (8/10) the IF was strong (Figs 1 and 2) and in a few cases (2/10) weak but clearly visible (Fig. 3). At the dermo–epidermal junction the IF seemed to coalesce with that of the basement membrane (Figs 1 and 2). In some samples the staining intensity was so strong that it could not be separated from that of the basement membrane, constituting an almost homogeneous broad IF band in the papillary dermis (Fig. 2).

Reticular dermis. Fibronectin was found in a fibrillar and linear distribution in the reticular dermis. The IF intensity was less pronounced than in the papillary dermis, but was increased in areas adjacent to subcutaneous tissue where a few speckled areas of IF probably represented staining of cellular elements. Dermal areas about epidermal appendages, such as pilosebaceous units (Figs 4 and 5) and eccrine sweat glands, demonstrated a much stronger IF with a fibrillar pattern. In the areas of pilosebaceous units some sections demonstrated circumscribed areas with a reticular staining in cross-cut sections (Fig. 6), whereas in other sections a more linear pattern was observed, probably representing longitudinally cut areas. These structures probably represented sections from arrector pili muscle.

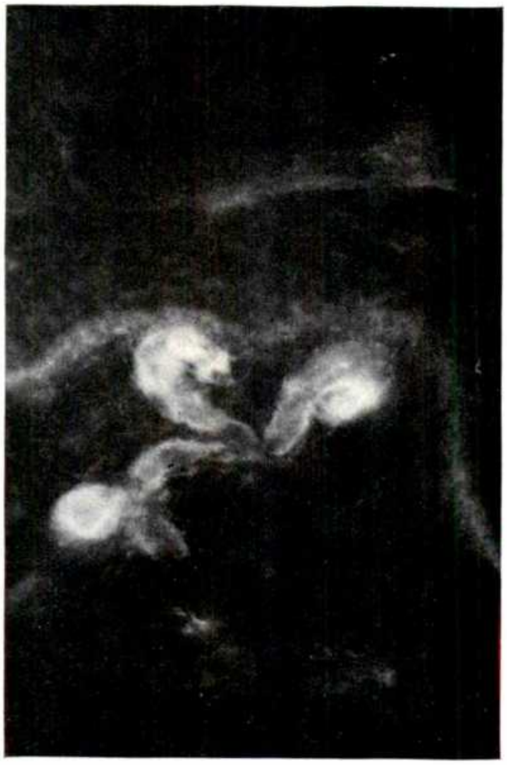
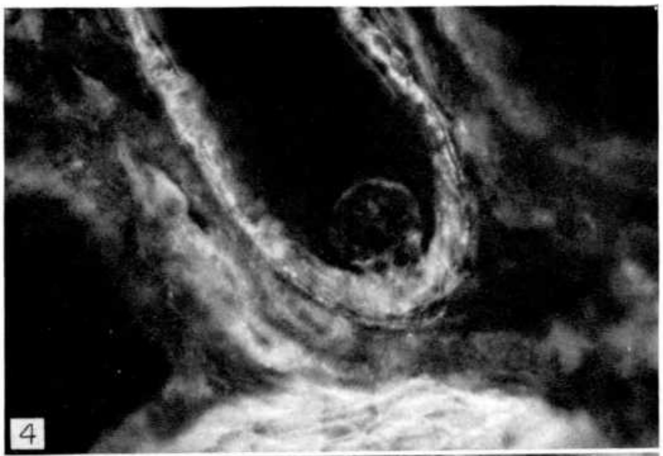


FIGURE 3. Staining of capillaries in the papillary bodies.

Vessels. The vascular system demonstrated a strong staining for fibronectin, usually including the entire vessel wall, but sometimes most pronounced against the vascular lumen indicating staining of endothelial areas. The strongest staining of vessels in the skin was found in the capillaries of the papillary bodies (Fig. 3) but staining of vessels was also observed in other parts of the skin.

Pilosebaceous units. As in the basement membrane of the dermo–epidermal region, the same kind of IF could be found around pilosebaceous units (Fig. 4). In some samples of cross-cut sections of the hair shaft, a fibrillar pattern of fibronectin was seen, probably in the area of the external root sheath (Fig. 5). Fibronectin was also found in the hair papilla (Fig. 4).

Eccrine sweat glands. Fibronectin is shown as a homogeneous annular structure in the peripheral parts of the glandular structure against the periglandular tissue, probably representing the dermo–epidermal junction area of this epidermal appendage. In these areas of the skin, fat globules from the



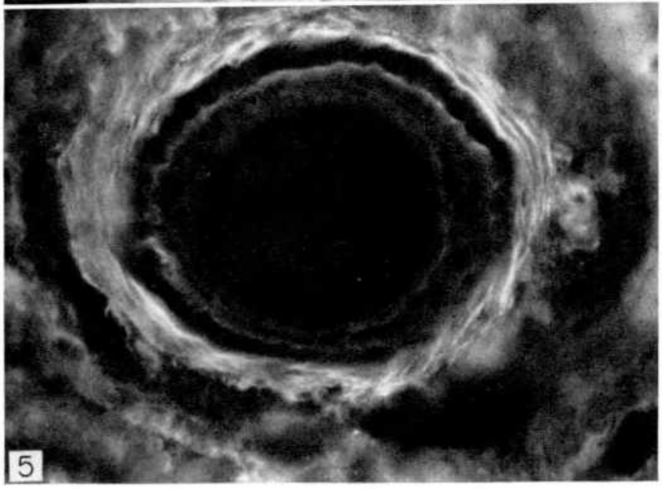


FIGURE 4. Staining of hair root sheath and of hair papilla.
FIGURE 5. Fibrillar staining of sections of cross-cut hair.

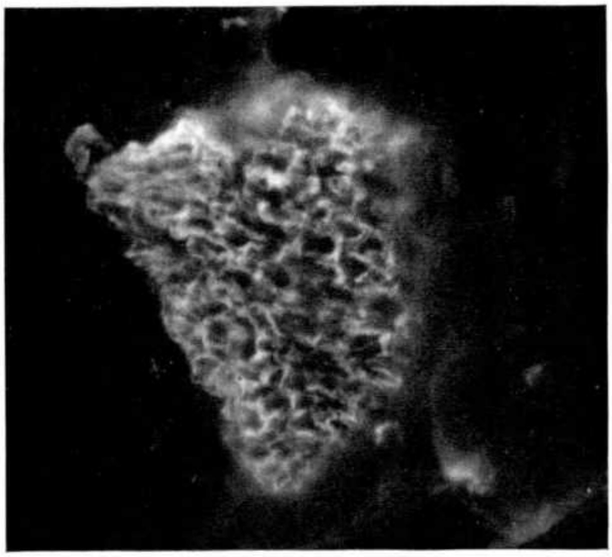


FIGURE 6. Reticular staining of cross-cut sections of errector pilli muscle.

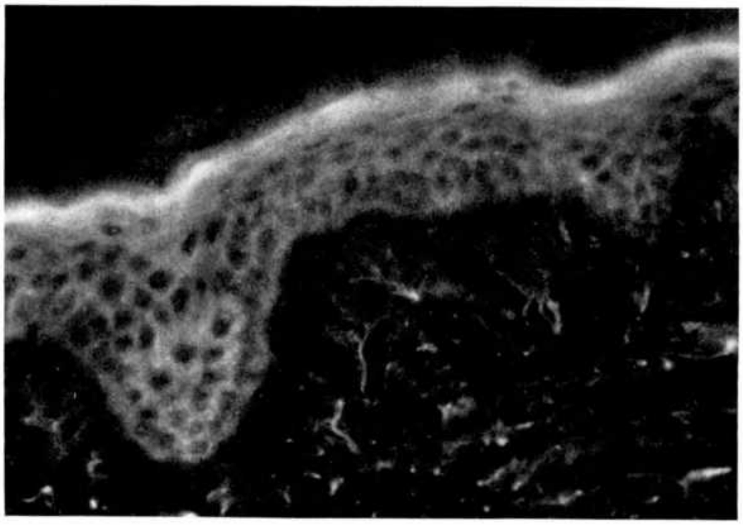


FIGURE 7. Control section with normal rabbit serum as the first layer.

subcutaneous tissue surround sweat glands and fibrillar elements of fibrinectin were found to continue from the dermis into the fat tissue.

Peripheral nerves. In peripheral nerves, fibronectin is demonstrated in the epineurium and in the perineurial sheaths in cross-cut and longitudinally oriented sections.

DISCUSSION

In the present study of normal human skin, fibronectin is mainly found in the basement membrane zone, in the papillary dermis and about epidermal appendages (pilosebaceous units and eccrine sweat glands). In the reticular dermis, fibronectin is present but to a minor degree and with a different type of distribution. In areas of the skin with much ground substance, such as in the papillary dermis, the IF pattern of fibronectin shows a reticular character, whereas in the reticular dermis larger bundles of fibronectin give a fibrillar pattern. These reticular and fibrillar patterns may be based upon the binding of fibronectin to collagen fibres. Both collagen and fibronectin demonstrate a very similar distribution (Klebe, 1974). The binding site of collagen to fibronectin lies between collagen residues 757 and 791 of the α-1 (I)-chain (Kleineman *et al.*, 1978) and is sensitive to collagenase. The demonstration of fibronectin in the dermo–epidermal junction area of the basement membrane is also seen in equivalent areas of hair, sebaceous glands and eccrine sweat glands.

Fibronectin is not detected in cultures of basal layer epithelial cells able to differentiate into epidermal cells (Sun & Chen, cited in Chen *et al.*, 1976), but has been demonstrated in cultured endothelial cells (Jaffe & Mosher, 1978), possibly representing the source of plasma-fibronectin (cold insoluble globulin). In the present study, no specific staining for fibronectin in epidermis of normal skin was observed. This is also the case in a number of different skin diseases investigated, with the exception of affected skin in psoriasis vulgaris where a prominent staining of fibronectin is observed above elongated dermal papillae in the stratum corneum areas, especially in Munro abscesses (unpublished data).

Fibronectin is present in the vascular system. This has previously been reported by Linder *et al.*

(1975) who demonstrated this glycoprotein in capillary walls and between vascular smooth muscle cells. In the present study fibronectin was found in the entire vascular wall, with a more prominent staining of endothelial areas against the vascular lumen.

Fibronectin has been demonstrated in sarcolemma of striated muscles (Linder *et al.*, 1975) and in smooth muscle cells (Vaheri *et al.*, 1977). In the present study, a localized reticular IF pattern has been observed in the reticular dermis in some skin sections, probably representing cross-cut sections of errector pili muscle.

In peripheral nerves, fibronectin is found in the epineurium and in the perineural sheaths. This is best demonstrated in cross-cut sections but can also be seen in longitudinally cut areas. These structures are known to contain longitudinally oriented collagen fibres.

In the light of our existing knowledge of fibronectin and of the present study this glycoprotein seems to represent an important aspect of the anatomy and physiology of the skin, with its prominent presence in such structures as the basement membranes of the dermo–epidermal junction and of the epidermal appendages (pilosebaceous units and eccrine sweat glands) of the vascular and nervous systems and in the connective tissue of all parts of dermis and subcutis. The role of fibronectin in the pathophysiology of the skin remains to be investigated.

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Juvenile dermatitis herpetiformis: an immunoelectron microscopic study

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SUMMARY

Three children with persistent maculopapular and urticarial lesions and vesicles at the predilection sites of dermatitis herpetiformis (DH) were shown to exhibit typical granular, papillary IgA and C3 deposits in the tips of the dermal papillae, as demonstrated by direct immunofluorescence. By immunoelectron microscopy, the IgA deposits were associated with the microfibrils of the elastic fibres as has been described in DH of the adult. C3 deposits were scattered throughout the papillary dermis.

Despite the similarity of the clinical appearance, history with regard to gluten sensitive enteropathy (GSE) varied in these three cases. In one child, the skin lesions appeared following faults in the gluten free diet on which he was kept for coeliac disease. Another child developed the skin lesions during a gluten free diet which was not strictly followed; no recurrences of gastrointestinal symptoms accompanied the eruption of DH. In the third case, no evidence for GSE in patient's history or in jejunal biopsies was present at the time of onset of DH.

Dermatitis herpetiformis (DH) is a (papulo)vesicular eruption, characterized by distinct histopathological (Eng & Moncada, 1974) and immunopathological criteria (Chorzelski *et al.*, 1971). The discovery of jejunal epithelial atrophy (Marks, Shuster & Watson, 1966), and the clearing of skin lesions under gluten free diet (GFD) (Fry *et al.*, 1973; Heading *et al.*, 1976; Reunla, 1978), have demonstrated the association of DH and gluten sensitive enteropathy (GSE). The conflicting reports on the immunopathology of childhood bullous disease and the difficulty in clinical classification of juvenile DH vs. juvenile bullous pemphigoid (Kim & Winkelmann, 1961; Jablonska *et al.*, 1971; McGuire & Nordlund, 1973; Bean & Jordon, 1974; Clayton & Feiwel, 1977; Esterly *et al.*, 1977; Van der Meer *et al.*, 1977) prompted us to report our results,

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PATIENTS AND METHODS

Clinical picture

The clinical picture of the three children (one male and two females) was essentially identical with regard to localization and morphology of the eruptions. Persistent maculopapular and urticarial lesions were found predominantly in the nuchal area, the shoulder and the buttock regions, and on the extensor surfaces of the extremities, especially the elbows and knees (Fig. 1a); lesions were sharply demarcated, erythematous, and exhibited tiny vesicles in herpetiform distribution (Fig. 1b). All children complained of severe itching.

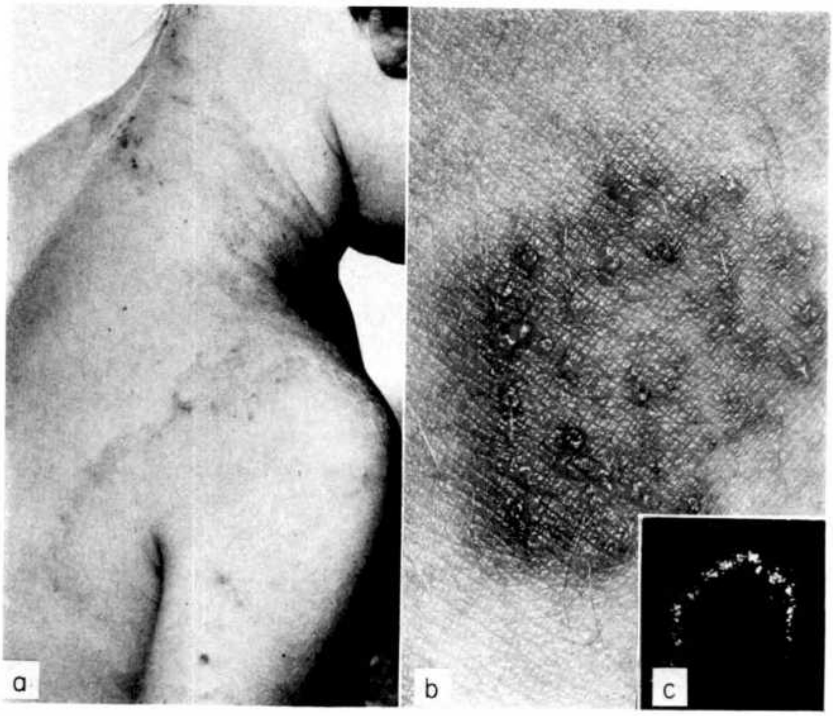

FIGURE 1. Case 1. 7-year-old boy. (a) Urticarial lesion with marked erythematous borders. (b) Detail from extensor surface of upper arm. Tiny, grouped vesicles on an erythematous base. (c) Perilesional buttock skin. Direct immunofluorescence. Goat anti human IgA conjugate; granular deposits in the tips of the dermal papillae.

History

In contrast to the similarity of the clinical picture, the history was different in the three children. In Case 1 (see Table 1) the history did not reveal any clinical signs of coeliac disease and absorption tests failed to give evidence of GSE at the time of the onset of the skin rash. In Cases 2 and 3, coeliac disease was diagnosed clinically and confirmed by jejunal biopsy when the girls were 8 months old. From this time both children were on a gluten free diet (GFD) and the clinical signs of GSE improved. In one child (Case 2), eruption of the skin lesions was observed following dietary faults. In Case 3 the dietary regimen was not followed strictly and, possibly as a consequence, a rash appeared.

TABLE 1. Details of patients

Case	Initials, age (years), sex	GSE*	HLA-B8	Indirect IF†	Direct IF granular pattern	Response to GFD‡
1	M.R., 7, male	No	Positive	Negative	IgA, C3	Good; clearing of skin lesions with diet
2	B.R., 7, female	Yes	Not done	Negative	IgA, C3	Good; clearing of skin lesions with diet
3	S.A., 3, female	Yes	Positive	Negative	IgA	Good; clearing of skin lesions with diet

* GSE: Gluten sensitive enteropathy.
† IF: Immunofluorescence.
‡ GFD: Gluten free diet.

Investigations

Details of patients are listed in Table 1. Laboratory investigations included determination of erythrocyte sedimentation rate, urinalysis, complete blood cell counts, clotting time, fasting blood sugar, SGOT, SGPT, alkaline phosphatase, LDH, serum bilirubin, serum electrolytes, serum iron, cholesterol, triglycerides, uric acid, blood urea nitrogen, serum creatinine and serum electrophoresis. All values were found to be within normal limits.

Treatment

All three children were kept on a carefully monitored GFD and treated symptomatically with oral antihistamines. Within 6–8 weeks the skin lesions improved in all three cases (both in the two children with coeliac disease and in the boy without evidence of GSE).

Biopsies

Tissue specimens were taken from perilesional buttock skin under local anaesthesia, by surgical biopsy, for routine haematoxylin eosin staining and direct immunofluorescence and, by shave biopsy with a razor blade, for immunoelectron microscopy. Serum samples were taken at the same time.

Immunofluorescence

Direct method. Tissue specimens were snap frozen in liquid nitrogen and processed for the detection of *in vivo* bound IgG, IgA, IgM and C3 using standard methods (Beutner *et al.*, 1973).

Indirect method. Serum samples were investigated for the presence of circulating auto-antibodies by indirect immunofluorescence using normal human skin and monkey oesophagus as substrates (Beutner *et al.*, 1973).

Horseradish peroxidase anti-horseradish peroxidase immunoelectron microscopy

Direct method. Skin specimens obtained by shave biopsy were processed for the ultrastructural demonstration of *in vivo* bound IgA and C3 as described previously (Holubar *et al.*, 1975). Controls were run in an additional set-up using normal goat serum as the first step of the immunological reaction chain.

Antisera (conjugates)

Commercially available conjugates (Hyland Laboratories, Costa Mesa, California, U.S.A.) were used (goat antisera to human Ig (polyvalent), IgG, IgA, IgM and C3). Details of the antisera used in the immunological reaction chain were described elsewhere (Holubar *et al.*, 1975).

RESULTS

Histopathology

In all three cases the typical histopathological picture of DH (Eng & Moncada, 1974) was observed including subepidermal splitting, neutrophil microabscesses in the tips of the dermal papillae and neutrophil infiltrates surrounding the blood vessels of the upper dermis. Few eosinophils were found scattered throughout the accumulations of neutrophils.

Immunofluorescence

Direct method: In all three cases intense fluorescent staining was found with anti-IgA conjugate (Fig. 1c); C3 was present in two cases (Cases 1 and 2) in weak intensity; IgG and IgM could not

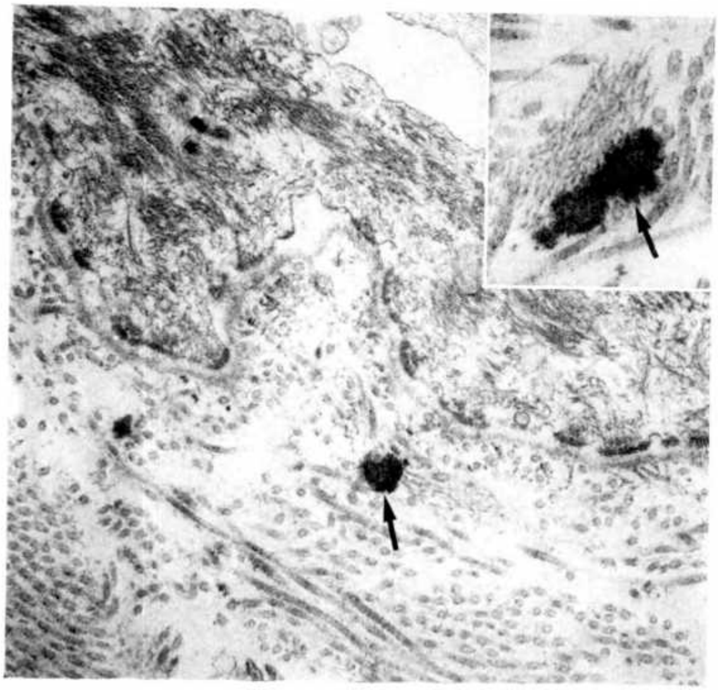

FIGURE 2. Case 2. Perilesional buttock skin. Horseradish peroxidase anti-horseradish peroxidase technique; goat antihuman IgA conjugate. Electron dense reaction product indicating the sites of IgA deposits which are distributed subepidermally and closely associated to bundles of microfibrils (inset) (× 20,000; inset: × 38,500).

be detected. The distribution of IgA and C3 was typical (Chorzelski *et al.*, 1971) with fine granular deposits, predominantly in the tips of the dermal papillae and in weaker intensity along the basement membrane zone.

Indirect method: No circulating autoantibodies could be demonstrated on normal human skin and monkey oesophagus as substrates using antihuman Ig (polyvalent) or anti-IgA conjugate.

Horseradish peroxidase anti-horseradish peroxidase immunoelectron microscopy

Direct method. The electron dense reaction product, visualizing *in vivo* bound IgA, was found in all three cases and was distributed throughout the uppermost strata of the papillary dermis near the dermo-epidermal junction, forming focal aggregates of irregular appearance (Fig. 2). At higher magnifications these aggregates were always localized beneath the basal lamina and the area of the anchoring fibrils. IgA deposits (Fig. 2, inset) appeared to be closely associated with bundles of microfibrils of the elastic fibres, whereas C3 deposits found in Cases 1 and 2, did not disclose such an obvious association to underlying structures (Fig. 3). C3 deposits usually appeared smaller in size and number, and of weaker intensity. Controls sections were free of reaction product.

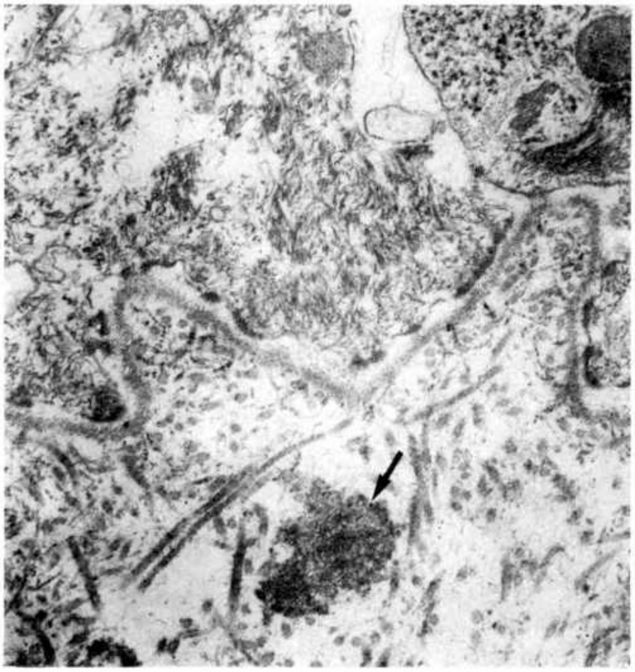

FIGURE 3. Case 2. Perilesional buttock skin. Horseradish peroxidase anti-horseradish peroxidase technique; goat antihuman C3 conjugate. Clumps of electron dense reaction product (arrow) marking the site of *in vivo* fixed C3 (× 29,000).

DISCUSSION

These three cases of juvenile DH seem to be of interest from three points of view.

(1) The clinical picture mimics chronic urticaria and epidermomycosis, and only careful examination

of the lesions revealed the microvesicles in the erythematous areas. These clinical manifestations are in contrast to most reports of juvenile DH which usually describe larger vesicular lesions or blisters (Kim & Winkelmann, 1961; Jablonska *et al.*, 1971).

(2) The response of DH to GFD by clearing of the skin lesions and the remission of intestinal atrophy is well established (Heading *et al.*, 1976; Reunala, 1978). The underlying pathological mechanism, expressed clinically as sensitivity to gluten protein, seems to be similar in DH and coeliac disease (Strober *et al.*, 1975; Katz & Strober, 1978). In our three cases the different onset of skin lesions with regard to the GSE—in one case during GFD which was not strictly followed; in the second during GFD but due to errors in the dietary regimen; and in the third without any evidence of GSE—raises speculations that additional mechanisms besides GSE might be decisive for the development of skin lesions.

(3) Considering the conflicting reports of immunofluorescent criteria of childhood bullous disease and the related difficulties in differential diagnosis, especially juvenile DH vs. juvenile BP, our present cases emphasize the identity of juvenile DH. Identical ultrastructural localization of the IgA deposits in DH in the adult and in our childhood cases make it likely that they represent one disease (Yaoita & Katz, 1976; Stingl *et al.*, 1976).

However, as in DH of the adult (Yaoita & Katz, 1976, 1977; Pehamberger *et al.*, 1977a, b), children exhibiting linear distribution of IgA along the basement membrane, with or without circulating IgA anti basement membrane zone antibodies, have been reported as 'bullous disease of childhood' (McGuire & Nordlund, 1973; Clayton & Feiwel, 1977; Esterly *et al.*, 1977; Van der Meer *et al.*, 1977). According to our own experience with such cases, the clinical picture does not allow any conclusions as regards type of IgA deposition (granular vs. linear; and lamina lucida vs. sublaminar), and regarding the class of Ig involved (IgG vs. IgA). Consequently linear types of Ig deposition, by immunofluorescence, should be subjected to immunoelectron microscopy for further classification. Inclusion into BP or DH groups respectively, of such linear cases, can only be made with certainty when classification of antibodies involved and identification of target sites in skin have been achieved.

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Corticosteroids, aurothioglucose and soybean trypsin inhibitor do not prevent pemphigus antibody-induced acantholysis *in vitro*

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SUMMARY

Hydrocortisone, triamcinolone acetonide, aurothioglucose and soybean trypsin inhibitor were added to normal human skin explants cultured with IgG from pemphigus serum to determine if acantholysis could be prevented. At the therapeutic concentrations used none of these compounds prevented binding of the autoantibody to the epidermal target cells, and none prevented acantholysis. These experiments support the concepts that the pemphigus antibody alone is responsible for producing the acantholytic lesions of pemphigus, that the therapeutic effectiveness of steroids and gold salts is probably due to their ability to reduce serum autoantibody titres and that pemphigus acantholysis is probably not caused by a serine proteinase.

Definitive evidence has been obtained from *in vitro* studies using normal human skin (Bellone & Leone, 1956; Schiltz & Michel, 1976; Michel & Ko, 1977) or monkey skin (Deng *et al.*, 1977) that the serum from pemphigus patients can cause the characteristic histological lesion of the disease. Schiltz & Michel (1976) demonstrated that it is the IgG from pemphigus serum which causes the epidermal damage. Electron microscopic studies have confirmed that the acantholysis produced in cultured human skin (Hu, Michel & Schiltz, 1978) or monkey skin (Barnett, Beutner & Chorzelski, 1977) is identical to that seen in spontaneous pemphigus lesions.

Two recent studies have suggested that pemphigus acantholysis results from the action of epidermal hydrolytic enzymes which are released or activated by keratinocytes following their interaction with the pemphigus antibody. Farb, Dykes & Lazarus (1978) showed that monolayer cultures of mouse epidermal cells became less adherent to the culture dishes when pemphigus serum was added to the culture medium. Based on their observation that this effect was prevented by serine protease inhibitors (soybean trypsin inhibitor and α_2-macroglobulin), they concluded that the loss of cellular adhesion and pemphigus acantholysis were caused by a lysosomal serine proteinase which was released by the cells.

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We demonstrated (Schiltz, Michel & Papay, 1978) that when the IgG fraction from pemphigus serum was incubated with suspensions of primary human skin epidermal cells, protein synthesis was inhibited, the cells were killed and solubilization of previously-insoluble cellular material occurred. This solubilization was shown to be caused by a pH 6·5 active non-lysosomal hydrolytic enzyme(s) from the epidermal cells.

In the present study we tested two corticosteroid derivatives to determine their effects on pemphigus antibody binding to epidermal cells and on acantholysis in cultured skin explants. Systemically administered corticosteroids are widely used for treating pemphigus. Although it seems likely that the major therapeutic effect of corticosteroids in pemphigus is to reduce serum autoantibody titres (Beutner *et al.*, 1973), no information is available concerning a direct role for these compounds in preventing acantholysis in the affected epithelial tissues. Similarly, gold salts have been used successfully as systemic agents for treatment of pemphigus, and Penneys *et al.* (1973) demonstrated that gold therapy results in a reduction of serum antiepithelial antibody titres. Penneys *et al.* (1974) speculated that gold salts may prevent pemphigus lesions because of their ability to inhibit selected epidermal enzymes. Later, however, Penneys *et al.* (1975) concluded that the gold concentrations attained in the epidermis of patients undergoing chrysotherapy were too low to cause inhibition of these enzymes, and that the effectiveness of the drug was probably not due to direct action on the epidermis. Finally, we have tested the effects of soybean trypsin inhibitor on acantholysis in order to evaluate the notion of Farb *et al.* (1978) that pemphigus acantholysis is caused by an epidermal serine proteinase enzyme.

MATERIALS AND METHODS

Normal human skin from patients undergoing radical mastectomy was used in these experiments. The preparation and culture of this tissue has been described (Schultz & Michel, 1976). The skin was cut to a thickness of 0·4 mm with a Castroviejo keratotome and small pieces (2 mm × 2 mm) were explanted with the dermal side down onto paraffin-edged 'rafts' of sterile lens paper (Sarkany, Grice & Caron, 1965). The 'rafts' were floated on the surface of the appropriate culture medium in 35 mm Falcon tissue culture dishes. The incubations at 37°C in a humid CO_2 incubator were carried out in an atmosphere of 5% CO_2/95% air. The culture medium was Ham's F-10 medium (Ham, 1965) containing twice the normal concentrations of amino acids and sodium pyruvate + 10% heat-denatured fetal calf serum + a mixture of antibiotic-antimycotic (Grant Island Biological Co.) + purified pemphigus IgG or control human IgG (Schiltz & Michel, 1976). The resulting protein concentration of the pemphigus IgG was 23 mg/ml and the anti-intercellular cement substance (ICS) titre was 160 as determined using monkey oesophagus as substrate. We have previously shown that minimal titres of 80 are required to produce acantholysis in these explants (Schiltz & Michel, 1978). Serum from normal individuals with blood type AB was the source of the normal IgG, since it is known that anti-blood group antibodies react to antigens located in the epidermis (Grob & Inderbitzin, 1967). The protein concentration of the normal IgG was adjusted to that of the pemphigus IgG. Hydrocortisone (Sigma Chemical Co., #H4001), triamcinolone acetonide (Sigma Chemical Co., #T6501) and aurothioglucose (Sigma Chemical Co., #A0632) were used in the culture media at final concentrations of 10 μM. These were considered to be at least equivalent to therapeutic doses, and are probably well in excess of the concentrations attained in the epidermis of patients receiving systemic corticosteroids or gold salts. It can be calculated that if 100 mg of hydrocortisone were administered systemically, and if this compound completely equilibrated with 40 l of body fluid, the maximal concentration in the body fluid would be approximately 7 μM. This concentration is unlikely to occur in the tissues, however, since the steroid will not equilibrate *in vivo*. Most corticosteroids are catabolized, and/or excreted into the urine, and it is known that the plasma half-life for hydrocortisone *in vivo* is about ½–1 h (Goodman

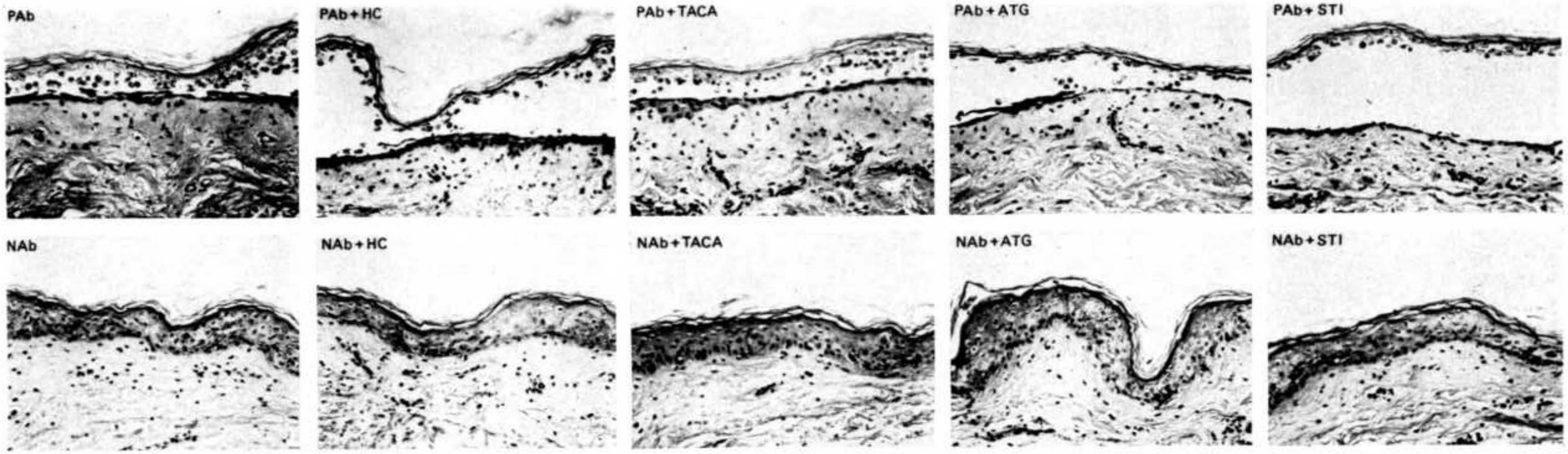

FIGURE 1. Normal human skin explants which had been cultured for 48 h in F-10 medium containing normal IgG (NAb) or pemphigus IgG (PAb), either alone or in the presence of 10^{-5}M hydrocortisone (HC), 10^{-5}M triamcinolone acetonide (TACA), 10^{-5}M aurothioglucose (ATG) or 100 μg/ml soybean trypsin inhibitor (STI) (H & E, × 200).

& Gilman, 1975). The calculation for gold concentration was more direct. Penneys *et al.* (1975) reported that the mean gold concentration in the epidermis of eight patients undergoing chrysotherapy (6·8 years average duration of therapy) was 1·45 ± 0·9 μg gold/g tissue wet weight. Using this value, it was calculated that the concentration of Au^+ in the epidermis would be 5·9 μM. Soybean trypsin inhibitor was tested at the same concentration (100 μg/ml) used by Farb *et al.* (1978) and was obtained from the same source (Sigma Chemical, #T9003). We tested this material for its ability to inhibit trypsin activity. At a final concentration of 100 μg/ml, it inhibited the capacity of twice crystallized trypsin (10 μg/ml, Grand Island Biological Co., #70680) to convert ^{3}H-labelled epidermal cell protein substrate (Schiltz *et al.*, 1978) to trichloroacetic acid-soluble radioactivity. During a 2 h incubation, solubilization was inhibited 84·1 ± 3·4%. In order to dissolve the steroids in the culture media, these compounds were first dissolved in heat-denatured fetal calf serum by gentle heating. The steroid-containing serum was added to the F-10 medium which contained the IgG antibodies described above. Direct immunofluorescence staining of cultured explants was performed using fluorescein-conjugated goat antihuman IgG (Hyland Labs) as previously described (Schiltz & Michel, 1976; Michel & Ko, 1977).

RESULTS

Skin explants were cultured for 3 days in F-10 medium containing normal IgG or pemphigus IgG, either alone or in the presence of the specific pharmacological compound being tested. Explants were removed at 5, 23, 48 and 72 h and examined by direct immunofluorescence or H & E staining for antibody binding and histology, respectively. Typical histological changes seen in the normal IgG controls during the period of culture were similar to those described previously (Schiltz & Michel, 1976). No changes were seen at 5 h. By 23 h, intercellular oedema was apparent and by 48 h some epidermal disorganization was apparent (Fig. 1). In addition, at 48 h epiboly (growth of the epidermis around the dermis) was common and a parakeratotic layer had begun to form. By 72 h parakeratosis was extensive, and all the other earlier changes were only slightly increased. Control cultures which contained normal IgG with hydrocortisone (HC), triamcinolone acetonide (TACA), aurothioglucose (ATG) or soybean trypsin inhibitor (STI) displayed histological changes which were similar to those described for explants grown in normal IgG alone. No acantholysis was observed in any of the control explants. The explants which had been cultured with pemphigus IgG during this same 72 h period were quite different from the controls. By 23 h, much more suprabasilar intercellular oedema had occurred and in some explants a suprabasilar split had developed. By 48 h, extensive acantholysis was apparent in all the explants which contained pemphigus IgG, including those grown in the presence of HC, TACA, ATG or STI (Fig. 1). The acantholytic cells which were produced were indistinguishable from those seen in spontaneous lesions from pemphigus patients (Civatte, 1943). These cells have small pyknotic nuclei surrounded by a clear zone or 'halo', an intensely eosinophilic cytoplasm and rounded borders. We could not discern differences either in the time of onset of acantholysis or in the degree of acantholysis in any of the explants which had been grown in pemphigus IgG, either in the presence or absence of the drugs.

To determine if the pemphigus antibody became bound to the epidermis, explants which had been cultured with pemphigus IgG were removed at selected times and examined by direct immunofluorescence. Antibody binding to the suprabasilar cells was seen in the 5 h cultures, and the intensity of the fluorescence was maximal in the 23 h explants. In those explants cultured with pemphigus IgG in the presence of HC, TACA, ATG or STI, the IgG also was bound and the fluorescence was not diminished when compared to the control pemphigus IgG. Binging of IgG did not occur in the epidermis of control explants cultured with normal IgG, either alone or in the presence of any of the compounds tested.

DISCUSSION

Corticosteroids and gold salts, when administered systemically, are effective in the management of pemphigus (Lever, 1972; Penneys *et al.*, 1973). Since treatment with these agents results in reduction of serum anti-epithelial antibody titres, and since the clinical severity of the disease is usually related to the auto-antibody titre (Beutner *et al.*, 1973), it is reasonable to assume that a major mode of action of these compounds is to inhibit autoantibody production. The question we have attempted to answer in this study is whether steroids or aurothioglucose, at therapeutic concentrations, will exhibit additional, more direct effects on the acantholytic process. This could occur by interfering with autoantibody binding to the epidermal target cells and/or by inhibition of one or more steps of the acantholytic process. Our results clearly show that these compounds prevent neither autoantibody binding nor acantholysis. The lack of effect of gold on antibody binding confirms and extends the studies of Penneys *et al.* (1974) which showed that aurothiomalate does not complex the pemphigus antibody or prevent binding of the antibody to frozen sections of human skin. In other studies we have determined that the corticosteroids also do not interfere with antibody binding to frozen skin sections (unpublished).

The rationale for testing the effects of soybean trypsin inhibitor on acantholysis is based on our belief that acantholysis is caused by epidermal hydrolytic enzymes (Schiltz *et al.*, 1978) and on the hypothesis of Farb *et al.* (1978) that acantholysis is caused specifically by an epidermal serine proteinase enzyme. Since acantholysis was not prevented by this inhibitor, we conclude that pemphigus acantholysis in human epidermis is not likely to be caused by a serine proteinase. This does not, however, rule out the possibility that a similar process in mouse skin, as reported by Farb *et al.* (1978) may be mediated by such an enzyme.

Finally, it should be pointed out that even though the corticosteroids and aurothioglucose did not prevent pemphigus antibody-induced acantholysis *in vitro* when tested at therapeutic concentrations, our experiments do not exclude the possibility that much higher concentrations attained by topical application or by intradermal injection may have beneficial effects on the pemphigus patient.

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8-MOP plasma levels in PUVA problem cases with psoriasis

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SUMMARY

8-MOP plasma levels of psoriatic patients poorly responsive to PUVA treatment (PUVA problem cases) ($N = 14$) and of psoriatic patients with adequate response to photochemotherapy ($N = 7$) were measured for 8 h after oral ingestion of 0·6–0·8 mg/kg body weight, using a gas chromatographic method.

We investigated whether there are any differences in the course of the plasma kinetics between the two groups. Problem patients showed significantly lower 8-MOP plasma levels than the control group. Furthermore, the 8-MOP plasma levels increased more rapidly in the control group than in these problem patients.

Deviations in time of the maximum 8-MOP plasma levels from the expected 2 h peak could be observed in 50% of the problem cases compared to only 14% of the control patients. There is no correlation between the dose and the 8-MOP plasma level achieved in the two groups, i.e. higher doses do not result in higher levels.

In individual cases there is not always a correlation between the plasma maximum of 8-MOP at the time of UV-A irradiation and the response to treatment.

Adjustment of the UV-A irradiation to coincide with the maximum plasma levels led to an improvement in therapeutic results for three problem patients.

Photochemotherapy of psoriasis is a widely accepted method of treatment. Many reports have been published concerning the technical and therapeutic results (Parrish *et al.*, 1974; Wolff *et al.*, 1975a, b, 1976; Hofmann, Plewig & Braun-Falco, 1976; Melski *et al.*, 1977; Weber, 1978).

The therapeutic efficacy is remarkably high and is said to be 88–93% (Melski *et al.*, 1977; Wolff *et al.*, 1977). Our unpublished data agree with these results.

The investigations of Pathak *et al.* (1975) on the metabolism of psoralens showed that cutaneous

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photosensitization by UV-A occurs maximally between 2 and 4 h after oral administration of 8-MOP. On the basis of these findings, the UV-A irradiation is given routinely 2 h after ingestion of 8-MOP.

However, 4 years' experience has shown that a few patients do not respond satisfactorily; why these patients fail to improve sufficiently remains unsolved. The purpose of this work was to monitor 8-MOP plasma levels to establish any differences or shifts in plasma maxima which might correlate with the therapeutic responses.

PATIENTS AND METHODS

Control patients

A group of our psoriatic patients (N = 100) with adequate response to oral photochemotherapy exhibited a 100% skin clearing after 20·1 ± 6·8 doses of radiation, corresponding to a total UV-A dose of 80·7 ± 55 J/cm². Seven patients from this group served as controls (Table 1).

Problem cases

These are defined as patients (N = 14) who do not show adequate response to the standard PUVA procedures. They are divided into four subgroups (Table 2).

Group 1. Patients who need more PUVA treatments and a higher total UV-A dose (40 PUVA treatments; 253·4 J/cm² UV-A total dose) in the initial treatment phase than the control group to clear psoriasis. These patients remained free from psoriasis during maintenance therapy.

Group 2. Patients with adequate response in the initial treatment phase (15·7 PUVA treatments; 52·75 J/cm² UV-A total dose) but rapid recurrence of psoriasis. During twice weekly maintenance therapy, psoriasis remains only 70–80% clear.

Group 3. Patients who need more PUVA treatments (33 PUVA treatments; 366 J/cm² UV-A total dose) during the initial treatment phase until psoriasis is cleared completely, compared to the control group. Furthermore, the psoriasis rapidly recurred and only a 70–80% improvement is obtained despite maintenance treatment twice a week.

Group 4. These patients have a higher number of treatments and total doses (45·5 PUVA treatments; 316·6 J/cm² UV-A total dose), but only exhibit a clearing of 70–80%. Twice weekly maintenance therapy effects only a final clearing of 70–80%.

Investigational procedure

All patients in this study had normal liver and kidney function. They received a uniform breakfast consisting of two rolls, 25 g of jam and 20 g of butter. One hour later venous blood was drawn as blank value. 8-MOP 0·6–0·8 mg/kg body weight (Meladinine, Basotherm, Germany) was ingested with a glass of water. At intervals of 1, 2, 3, 4, 6 and 7–8 h after oral administration 5 ml samples of venous blood were drawn from the antecubital vein with a heparinized syringe. The blood was immediately centrifuged and the plasma stored at −20°C. The plasma was handled and stored in the dark.

The plasma concentration of 8-MOP was determined by means of gas chromatography as described by Schmid & Koss (1978), using 0·1–0·5 ml plasma for analysis. The reproducibility (standard deviation) is about 2% over this concentration range. The lowest limit of detection is about 10 ng/ml.

Time-adjustment of UV-A irradiation to coincide with the maximum plasma level of 8-*MOP*

Following the determination of the 8-MOP plasma levels the time at which UV-A radiation was

TABLE 1. Clinical data of responsive patients with psoriasis under PUVA therapy

					Initial therapy			Maintenance therapy	
Patient	Sex	Age (years)	Type of psoriasis	Skin type	Percentage of clearance	Total dose of UV-A (J/cm²) up to complete clearance	No. of treatments up to complete clearance	No. of treatments per week	Percentage of clearance
1	f	72	p.pl.	III	100	73	22	1	100
2	m	50	p.pl.	III	100	116	23	1	100
3	f	70	p.pu.	II	100	37	17	1	100
4	m	36	p.pl.	III	100	63	22	1 per 2 weeks	100
5	m	68	p.pl.	III	100	110	25	1	100
6	m	61	p.pl.	IV	100	88	24	1 per 2 weeks	100
7	m	21	p.e.*	III	100	78	22	1	90–100 (skin lesions); 100 (improvement of arthropathy)
						Mean dose 80·7	Mean no. 22		

p.pl. = plaque psoriasis.
p.pu. = pustular psoriasis.
p.e. = erythrodermic psoriasis.
* psoriatic arthritis.

TABLE 2. Clinical data of PUVA problem cases with psoriasis

						Initial therapy			Maintenance therapy	
Patient	Group	Sex	Age (years)	Type of psoriasis	Skin type	Percentage of clearance	Total dose of UV-A (J/cm²) up to maximum clearance	No. of treatments up to maximum clearance	No. of treatments per week	Percentage of clearance
8	I	f	36	p.pu.	III	100	239	40	1	100
9		m	41	p.pl.	III	100	268	40	1	100
10	II	f	59	p.gu.	III	100	65	22	2–3	70–80
11		m	40	p.e.	II	100	39·4	13	2	80
12		f	48	p.e.	III	100	56·4	15	2	80
13		m	41	p.gu.	IV	100	51·5	13	2	80
14	III	m	38	p.pl.	III	100	407	36	2	85
15		f	54	p.pl.	III	100	326	30	Irregular intervals	70–90
16	IV	m	79	p.pl.	II	80	276	82	2	60–80
17		m	42	p.pl.	IV	80	734	42	2	60–80
18		f	80	p.e.	IV	90	183	39	2	80–90
19		m	39	p.pl.	III	90	318	35	2	70–80
20		f	38	p.pl.	III	90	170	40	2	70
21		m	39	p.gu.	III	90	219	33	2	80

p.pu. = pustular psoriasis.
p.pl. = plaque psoriasis.
p.gu. = guttate psoriasis.
p.e. = erythrodermic psoriasis.

applied was adjusted to coincide with the maximum 8-MOP plasma levels. The commonly accepted 2 h interval between oral intake of 8-MOP and UV-A treatment was extended for three of the problem patients to a 3 h interval to obtain improved treatment responses.

Statistics and calculations

The plasma levels of the control group were compared with those of the problem cases using Student's '*t*'-test for unrelated samples as a descriptive method.

The values for the area under the curve (AUC) were calculated by using the trapezoid rule.

RESULTS

Tables 3 and 4 show the values for plasma level and the corresponding AUC in responsive patients and problem patients. In the control group the 8-MOP plasma levels are higher than in the problem patient group (Fig. 1). The areas under the curves differ significantly between the two groups. The 8-MOP plasma level increases more rapidly in the control group than in the problem patients (Fig. 1). The decline of the 8-MOP plasma levels between 3 and 6 h after intake of 8-MOP is similar in the two groups. The maximum 8-MOP plasma level is not achieved in all patients 2 h after administration of the photosensitizing drug, the time at which UV-A irradiation is usually applied (Tables 3 and 4). Deviation in the control group occurred in only one patient, whose maximum 8-MOP plasma level was attained as late as 4 h after ingestion of the drug. In the problem cases time deviations were observed in seven of the fourteen patients. The distribution of the 8-MOP plasma level was as follows: three patients at 1 h, three patients at 3 h and one patient at 4 h after ingestion of 8-MOP. The other seven patients showed maxima at 2 h.

There is no correlation in either group between the applied 8-MOP dose/kg body weight and the height of the maximum and the AUC calculated (Tables 3 and 4). Examples are Patients 6 and 15 who achieved strikingly different 8-MOP plasma levels despite an identical 8-MOP dose of 0·67 mg/kg body weight.

For some patients in the two groups there is no apparent correlation between the height of the 8-MOP plasma level at the time of irradiation and the response to treatment. Examples are Patients 1 and 2 as well as 16, 17 and 18 (Tables 3 and 4).

TABLE 3. 8-MOP plasma levels in psoriatic patients with adequate response to PUVA therapy 1–8 h after oral intake of 8-MOP (responsive patients)

Patient	8-MOP dose (mg/kg body weight)	8-MOP plasma level (ng/ml)						Area under the curve (AUC) (ng/ml × h)
		1	2	3	4	6	7–8 h	
1	0·59	—	5	119	149	80	60	567·5
2	0 61	39	66	35	15	5	—	172
3	0 62	146	185	106	63	35	n.d.	601·5
4	0·63	0	110	91	52	10	17	316
5	0·64	112	124	68	46	19	10	421
6	0·67	151	289	248	179	157	73	1343·5
7	0·68	68	183	151	143	56	30	758·5

n.d. = not done.
— = not detectable.

TABLE 4. 8-MOP plasma levels in PUVA problem cases with psoriasis 1–8 h after oral intake of 8-MOP

Patient	Group	8-MOP dose (mg/kg body weight)	8-MOP plasma level (ng/ml) 1	2	3	4	6	7–8 h	Area under the curve (AUC) (ng/ml × h)
8	I	0·74	63	68	36	20	11	—	219
9		0·65	56	35	37	10	4	7	158
10	II	0·80	21	41	195	116	42	11	526
11		0·53	—	—	100	100	50	10	360
12		0·63	71	57	18	7	—	—	156·5
13		0·63	5	76	60	70	10	5	271
14	III	0·60	30	94	47	27	6	0	223
15		0·67	—	—	53	57	8	—	154·5
16	IV	0·65	23	106	93	66	17	8	363
17		0·63	151	104	54	41	16	12	414·5
18		0·71	5	187	166	77	13	15	514·5
19		0·68	—	—	—	—	—	—	0
20		0·58	44	58	63	41	n.d.	n.d.	226·5
21		0·63	5	46	36	17	0	0	112·5

n.d. = not done.
— = not detectable.

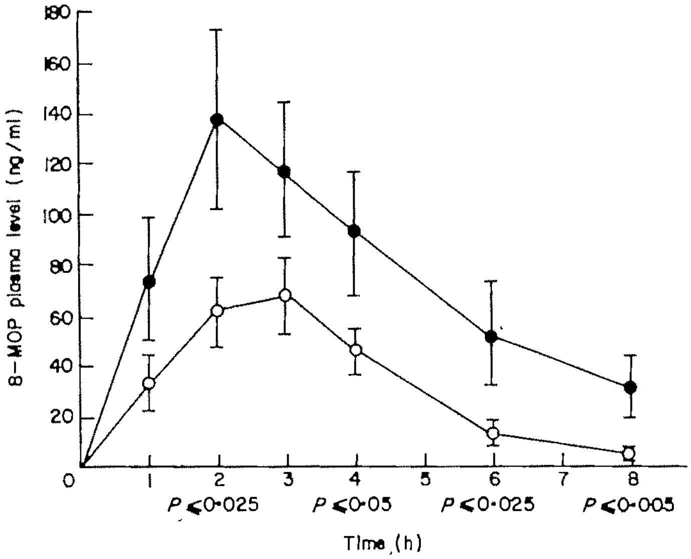

FIGURE 1. 8-MOP plasma levels in responsive patients (control group) and in problem patients with psoriasis (mean ± s.d.). (●) responsive patients; (○) problem patients.

Improvement of skin lesions could be obtained by shifting the UV-A irradiation to the peak of the 8-MOP plasma level in three of the problem cases (Patients 10, 11 and 15). The previous clearance rate of 70–80% could be raised to 90–100% without altering the UV-A dose or the frequency of treatments.

DISCUSSION

The group of the problem patients exhibited lower average 8-MOP plasma level maxima and AUC values than the control patients although the 8-MOP dose administered to the two groups was very similar (0·66 and 0·62 mg/kg body weight, respectively). Furthermore, the 8-MOP plasma level increased more rapidly in the control group than in the problem cases. One possible explanation for these two phenomena could be differences in absorption of the substance in the gastro-intestinal tract.

In accordance with other investigators we found that the time interval between 8-MOP ingestion and plasma peak is variable (Steiner *et al.*, 1977).

It is noteworthy, however, that deviation of the maximum 8-MOP plasma level from the expected 2 h peak occurred in 50% of the problem patients, but in only 14% of the control patients. As our results have shown, there is no correlation between the 8-MOP dose taken and the plasma level attained. These findings agree with those of other investigators (Steiner *et al.*, 1978).

We have tried to standardize the technical details of the investigational procedure by giving a 'standardized' breakfast. Furthermore, we controlled the uniformity of the 8-MOP tablets, which showed a maximum variation of 2·5%.

The purity of 8-MOP used was found to be 99·9%. We cannot support the findings of Hensby *et al.* (1978) who found a greater than expected inconsistency in the 8-MOP content of tablets from the same batch.

In certain cases there was no strong correlation between the maximum amount of 8-MOP in the plasma at the time of UV-A irradiation and the response to treatment. This is also in accordance with other investigators (Thune & Volden, 1977). Possible explanations include the lack of correlation between plasma and skin concentrations of 8-MOP or a lack of correlation between the UV-A dose applied and the UV-A dose attained in the skin.

The observation that considerable improvement of the skin lesions could be obtained by shifting the time of UV-A irradiation to coincide with 8-MOP plasma maxima, indicates that inadequate responses are probably due to low 8-MOP levels in plasma and skin during irradiation.

Little information is available as to whether the absorption characteristics of 8-MOP are the same from day to day in any individual patient. We have studied four subjects twice in this respect approximately 4 weeks apart with similar results.

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Psoralen photochemotherapy of psoriasis

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SUMMARY

The value of PUVA therapy in selected patients with severe psoriasis has been confirmed. Suppression of the disease was incomplete in those patients whose disease was unstable, with associated seborrhoeic dermatitic features.

No consistent change in the proportion of epidermal cells in DNA synthesis could be demonstrated during regression of the psoriasis as a result of PUVA therapy.

Photochemotherapy using oral 8-methoxypsoralen (8-MOP) and long wave ultraviolet radiation (PUVA) has been shown to suppress psoriasis and is being used increasingly in the treatment of the disease (Parrish *et al.*, 1974; Wolff *et al.*, 1976; Roenigk & Martin, 1977; Lakshmipathi *et al.*, 1977; Melski *et al.*, 1977). Until the potential long-term risks such as mutagenicity, ocular, hepatic and skin damage can be assessed, many physicians will restrict PUVA therapy to patients with extensive psoriasis or to those in whom the response to conventional treatment has been unsatisfactory. This is the policy currently being pursued by the present authors.

The mode of action of PUVA therapy in suppressing the disease is uncertain. Inhibition of DNA synthesis and cell division has been suggested as one possible mechanism (Walter *et al.*, 1973; Epstein & Fujukama, 1975). In this article we describe the results of serial estimations of epidermal cells in DNA synthesis during the clinical regression of psoriasis induced by PUVA therapy.

PATIENTS AND METHODS

Nine hospitalized patients, seven males and two females aged 33–71 years, gave their informed consent to participate in this study. The psoriasis of each subject was of many years duration affecting from 30% to 100% of the skin. In five patients the disease was in a non-eruptive stage, based on clinical criteria and cutaneous reactions to ultraviolet radiation (Hodgson & Hell, 1972); the remaining four had eruptive psoriasis, two exhibiting isomorphic reactions and one, universal disease with associated sero-negative arthritis. Previous treatments included oral corticosteroids, systemic methotrexate, coal tar, dithranol, dilute clobetasol propionate ointment, salicyclic acid and various emollients. For the purposes of this study, all these treatments were withdrawn at least 2 weeks prior to the initial

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biopsy examination and start of PUVA therapy; equal parts of White soft paraffin B.P. and Liquid paraffin B.P. were used as an emollient during the study. No other topical medicaments were allowed; several patients received Nitrazepam tablets B.P. at night and the arthritic subject was allowed non-salicylate analgesics. The following laboratory studies were performed before commencement and on completion of the study: haemoglobin estimation, white blood cell count, erythrocyte sedimentation rate, serum biochemical profile and urinalysis.

Ten milligram capsules of 8-MOP (Methoxsalen-Elder) were given according to the method of Parrish *et al.* (1974). The dosage varied between 20 and 50 mg and the tablets were administered 2 h prior to UV-A exposure. A UV-A Treatment Booth (Derma Control Inc., Dolton, Illinois, U.S.A.) consisting of fifty-six fluorescent tubes emitting high-intensity UV-A of 320–400 nm with a peak emission of 365 nm was used throughout the study. Phototesting and dosimetry were carried out in the manner described by Wolff *et al.* (1977); the minimal phototoxic dose (MPD) of UV-A was determined for each subject, except in the case of the patient with generalized psoriasis, prior to the commencement of the study. The initial exposure to UV-A was determined according to each patient's skin type and the MPD. Dosages and exposure times were regulated by a computerized control system (Derma) within the booth, the UV-A being recorded by an International Light (IL) photocell CM232 and measured on an IL 844 meter. Occasional independent checks of the UV-A output were made using a Blak-Ray UV meter J221. Two or three treatments were given each week, dose increments depending on the MPD, erythema reaction and degree of pigmentation of the clinically uninvolved skin. Recommended precautions regarding patient eye protection were followed; the UV penetration of goggles worn within the booth was less than 0·03 mW/cm^2.

Epidermal biopsy specimens (Arthur & Shelley, 1959) were taken under local anaesthesia (lignocaine) prior to the start of PUVA therapy and at intervals during treatment, in all cases 36 h after the preceding exposure to the UV radiation. Psoriatic plaques on the posterior lower trunk were selected for biopsy examination and the clinically uninvolved skin as far distant from the active lesions as possible was similarly examined. Each skin specimen was incubated at 37°C for 4 h in 1 ml of Hanks balanced salts solution and calf serum 7:3 containing tritiated thymidine 1 μCi/ml (360 μCi/μm). Fixation and processing for autoradiography followed conventional lines (Hell & Hodgson, 1966). Two thousand epidermal cells were counted on each specimen and the proportion of those showing more than twice the background number of grains calculated.

RESULTS

The clinical response to PUVA therapy is detailed in Table 1 according to the following scheme: 0—thick, erythematous, scaly lesions; 1—thick, erythematous, non-scaly lesions; 2—slightly palpable, erythematous, non-scaly lesions; 3—non-palpable, erythematous, non-scaly lesions; 4—hyperpigmented but otherwise macroscopically normal skin.

Of seven patients whose psoriasis improved, five cleared completely following eight to twenty-two treatments and total dosages of UV-A between 19 and 88 J/cm^2. Two additional patients showed significant partial regression of their disease after receiving 193 J/cm^2 (thirty-eight treatments) and 155 J/cm^2 (thirty-five treatments). Both these subjects continued treatment after termination of the trial; complete regression of the disease followed total dosages of 263 and 244 J/cm^2. PUVA therapy was discontinued in the one patient with universal psoriasis because of increasing claustrophobia within the treatment booth although the psoriasis had undergone significant improvement. In one additional patient PUVA therapy could not be tolerated after acute generalized pustular psoriasis had supervened.

The results of the estimations of the proportions of epidermal cells in DNA synthesis before and

TABLE 1. Clinical response to PUVA therapy

				Clinical features				
Sample no.	Patient	Sex	Age (years)	Activity	Extent (%)	Total dose (J/cm²)	No. of treatments	Clinical response
Complete clearing								
1	A.J.	M	33	Non-eruptive	60	—	—	—
2						11	4	1
3						30	10	2
4						52	16	3
5						88	22	4
6	K.T.	M	44	Non-eruptive	60	—	—	0
7						5	2	1
8						27·5	9	2
9						53·5	15	4
10	W.R.	M	60	Eruptive	90	—	—	0
11						6·5	3	2
12						19	8	4
13	W.H.	M	69	Non-eruptive	30	—	—	0
14						10	3	1
15						16	6	2
16						24	9	3
17						36	12	4
18	H.K.	M	71	Non-eruptive	50	—	—	0
19						11·5	9	2
20						48	18	4
Partial clearing								
21	D.W.	F	34	Non-eruptive	40	—	—	0
22						3·5	3	0
23						15·5	8	0
24						47	17	1
25						95	26	2
26						193	38	2
27	F.W.	M	36	Eruptive	70	—	—	0
28						3·5	3	0
29						16	9	1
30						47	18	1
31						96	27	2
32						155	35	3
33	A.B.	M	44	Eruptive	—	—	—	1
34						6	4	2
35						12	7	1
36	V.W.	F	56	Eruptive and arthritis	100	—	—	1
37						0·5	1	2
38						2	3	2
39						6	6	2
40						11	9	2
41						17	12	3

TABLE 2. Change in the proportion of cells in DNA synthesis during PUVA therapy

Sample no.	Patient	Sex	Age (years)	Activity	% Positive uninvolved	% Positive involved	Clinical response
Complete clearing							
1	A.J.	M	33	Non-eruptive	0·7	1·2	0
2					0·7	1·8	1
3					1·1	0·9	2
4					2·6	1·1	3
5					2·3	5·2	4
6	K.T.	M	44	Non-eruptive	2·5	3·4	0
7					0·8	18·0	1
8					0·8	2·4	2
9					1·6	1·6	4
10	W.R.	M	60	Eruptive	1·1	2·8	0
11					3·5	5·5	2
12					3·5	6·1	4
13	W.H.	M	69	Non-eruptive	3·9	5·1	0
14					2·0	2·8	1
15					1·9	3·9	2
16					1·5	2·7	3
17					1·9	1·3	4
18	H.K.	M	71	Non-eruptive	2·7	3·3	0
19					1·4	2·1	2
20					2·3	3·7	4
Partial clearing							
21	D.W.	F	34	Non-eruptive	1·8	1·6	0
22					2·2	3·7	0
23					1·5	2·4	0
24					1·4	1·6	1
25					1·3	0·7	2
26					1·8	1·1	2
27	F.W.	M	36	Eruptive	1·5	3·6	0
28					1·1	4·0	0
29					0·7	1·0	1
30					2·6	2·6	1
31					3·2	2·6	2
32					1·6	1·8	3
33	A.B.	M	44	Eruptive	1·1	1·6	1
34					2·4	4·7	2
35					4·7	1·6	1
36	V.W.	F	56	Eruptive and arthritis	—	2·3	1
37					—	2·2	2
38					—	3·4	2
39					—	8·3	2
40					—	1·7	2
41					—	4·4	3

during PUVA therapy in the involved and clinically uninvolved skin are shown in Table 2. Neither in the group of patients whose psoriasis underwent complete clinical regression nor in the two patients whose disease partially cleared was there any consistent reduction in the proportion of DNA-synthesizing cells of the involved or clinically uninvolved epidermis.

In six out of the nine patients, the proportion of epidermal cells actually increased during PUVA therapy, though with continuing treatment, the numbers decreased again in most. This pattern occurred in both involved and uninvolved skin and may be just the usual epidermal response to continuing injury, in this case, ultraviolet radiation.

DISCUSSION

The clinical results of this trial of PUVA therapy and of a larger group of patients treated in the same manner but who did not undergo biopsy examinations (Hodgson, unpublished data), confirm the many previous reports, some of which are quoted above, of the efficacy of this form of treatment in psoriasis. Of those patients reported in this paper, five (55%) experienced complete clinical regression of their psoriasis, and in three (33%) there was significant improvement. One patient failed to benefit from photochemotherapy, developing generalized pustular psoriasis which proved intolerant to further UV-A. These results were particularly gratifying in view of the fact that all patients in this study had suffered from severe extensive psoriasis of long duration, necessitating frequent hospitalization and exhibiting resistance or intolerance to conventional topical medicaments. Many had received both topical and oral corticosteroids in the past; several had required anti-mitotic therapy. In patients whose psoriasis reactivated on withdrawal of methotrexate, the disease was brought under control again with PUVA therapy. No side-effects of 8-MOP or UV-A were encountered, apart from slight nausea and some irritation of the treated skin. Maintenance therapy is being carried out in the majority of the patients.

The impression gained was that there is a group of patients who respond to relatively small or moderate dosages of PUVA therapy whereas other patients require much larger amounts of radiation to suppress the disease. This is illustrated in the present small series of patients; in the case of the five whose psoriasis cleared completely, the number of treatments and total dosages of UV-A required were markedly less than in the two patients whose disease had not cleared when the trial was terminated. It is the authors' impression, based on the treatment of twenty patients with psoriasis, that the response to photochemotherapy is more rapid when the disease is in a non-eruptive stage. In those patients whose psoriasis is labile and especially when seborrhoeic features are also present, the disease not only requires a larger number of treatments and greater total dosages of UV-A to achieve complete suppression, but also more frequent maintenance therapy.

We were unable to demonstrate any consistent decrease in the proportion of epidermal cells in DNA synthesis as a result of this treatment. Instead, our observations generally showed an initial increase which tended to disappear with continuing treatment. This is in accordance with our observations previously (Hell & Hodgson, 1966) where clinical clearing of psoriasis resulted in a slight increase in the proportion of cells in DNA synthesis after a variety of treatments. Where treatment has been reported to result in a decrease (Walter *et al.*, 1973) this may be explained by the skin used for testing, namely hairless mouse skin, and the conditions of the experiment, in this case, *in vitro* in a completely synthetic medium without serum. Damage shows more quickly in an inadequate medium: dying cells lose their capacity for DNA synthesis early (Houck, Weil & Sharma, 1972). Epstein & Fukuyama (1975) observed a depression in the number of epidermal cells synthesizing DNA within 6 h of a single large dose of UV-A to mouse skin painted with a solution of 8-MOP. Serial examinations showed recovery after 24 h and an increase in the number of cells in DNA synthesis 48 h after irradiation. The effect of PUVA therapy on epidermal DNA synthesis observed in our patients was a cumulative one and any comparison with the animal experiments involving a single exposure to 8-MOP and UV-A should be made with caution.

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Histological changes produced in skin by equally erythemogenic doses of UV-A, UV-B, UV-C and UV-A with psoralens

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SUMMARY

The sequential light microscopic histological changes produced in human skin by a single exposure of UV-A, UV-B, UV-C and oral 8-methoxypsoralen plus UV-A (PUVA) causing approximately equal degrees of delayed erythema response, have been evaluated. UV-C and UV-B affect the epidermis to a greater degree than UV-A, while UV-A affects the dermis to a greater degree than UV-B and UV-C. PUVA has prominent effects on both epidermis and dermis, differing in degree from those changes induced by UV-A, UV-B, and UV-C and are longer lasting. The sequence of histological changes following UV exposure is completed more rapidly after exposure to shorter UV wavelengths.

Increased recognition and definition of both beneficial and harmful effects of ultraviolet radiation create a need for quantification of individual components of skin reaction to various wavebands. Histological alterations are recorded as part of many dermatological investigations but these observations suffer from not being well correlated with careful dosimetry or with other parameters of phototoxicity such as delayed erythema. Erythema is an easily observed aspect of the delayed inflammatory response following ultraviolet irradiation of human skin and is the most widely used endpoint in dermatological photobiology. The human erythema action spectrum, the spectral power distribution of sunlight at the earth's surface, and absorption of UV by air have been arbitrarily used to define three wavelength bands of ultraviolet radiation (UV): UV-C (200–290 nm), UV-B (290–320 nm) and UV-A (320–400 nm). The minimal energy required to produce delayed erythema and the slope of the dose response curve differ for each of these portions of the ultraviolet spectrum (Hausser & Vahle, 1927; Everett, Sayre & Olson, 1969).

Miescher (1957) was the first to observe that the relatively non-erythemogenic UV-A primarily caused injury to dermal capillaries, with necrosis of endothelial cells. This was contrasted with the

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striking epidermal cytotoxic changes caused by irradiation of shorter wavelengths. However, Miescher (1957) did not follow the sequence of these changes or compare equally erythemogenic exposures of different wavelengths. Few observations have been made since that time comparing the histological changes induced by different portions of the UV spectrum. These reports are compromised by the absence of serial biopsies, by the use of massive exposure doses, by inadequate information about the degree of the erythema response, or by absent or inaccurate spectral radiometry.

The purpose of this paper is to describe and compare the sequential histological changes produced in human skin by doses of the different portions of the UV spectrum causing similar degrees of delayed erythema. In the case of UV-A, the changes occurring with and without the photosensitizer oral 8-methoxypsoralen are compared.

MATERIALS AND METHODS

Subjects

The untanned skin of the lateral lower back, at or beneath the belt-line, of eleven healthy fair-skinned Caucasians was used. These were three females and eight males with ages ranging from 20 to 40 years. Informed consent was obtained.

Radiation sources

UV-A. The output of a 1000 W xenon/mercury arc lamp (Hanovia 977 B–1) was collimated at f/1·0 by a quartz doublet lens, reflected from a dichroic mirror maximized for reflectance at 365 nm, and passed through Corning 7-51 and Schott WG 335 (2 mm) filters to provide intense UV-A in the virtual absence of other wavelengths. UV-A irradiance was 35 mW/cm^2 over a 5 cm × 5 cm square field, with a uniformity of ±8%. The emission spectrum was from 320 to 405 nm, with approximately 80% of the total power at 365±5 nm. The UV-B irradiance produced by this system was less than 0·1 μW/cm^2.

UV-B. A bank of four fluorescent FS40 T12 Westinghouse sunlamps with UV emission from 285 to 400 nm and a broad maximum at 313 nm was used. The erythema response to these lamps is almost entirely due to UV-B wavelengths (280–320 nm). The 295–305 nm irradiance was measured to be 200 μW/cm^2 at 15 cm from the lamps; at this same distance, the 280–320 nm (UV-B) irradiance was calculated to be 690 μW/cm^2 based on the spectral power distribution of the FS40 T12 lamp.

UV-C. Two Westinghouse WL 782-30 low-pressure mercury discharge lamps were used. Approximately 90% of the radiant emission of these lamps is 254 nm radiation. UV-C irradiance was 1·18 mW/cm^2 at 23 cm. The uniformity of irradiance for the UV-B and UV-C exposures was approximately ±6%.

Psoralen plus UV-A (PUVA). 8-methoxypsoralen was administered orally (0·6 mg/kg) and subjects were exposed exactly 2 h after ingestion to a high-intensity fluorescent UV-A source (emission spectrum 320–400 nm) described in detail previously (Levin & Parrish, 1974). The UV-A irradiance was 8 mW/cm^2 at 15 cm. The spectral emission of these lamps has a broad maximum at 355 nm and extends from 320 to 400 nm.

Radiometry

All irradiance measurements were performed using calibrated cosine-corrected detectors at the same distance from the lamps as used during cutaneous exposures. The irradiance produced by the UV-A sources was measured using an International Light, Inc., Model IL 442 radiometer which responds to 315–400 nm radiation with peak response at 365 nm.

The irradiance for the UV-B and UV-C sources was measured with an International Light, Inc., Model IL 700 radiometer system. The UV-C detector used was a model SEE-400 photodiode with response from 200 to 700 nm and 10 nm half power bandwidth, blocked to 10^{-4} transmittance outside the passband. UV-B irradiance was measured with the same photodiode fitted with an interference filter with maximum transmission at 297 nm and a half-power bandwidth of 10 nm, also blocked to 10^{-4} transmittance outside the passband. Calibrations were traceable to the U.S. National Bureau of Standards. A deviation of less than 1% in radiometer calibration was observed before and after irradiance measurements.

Erythema grading

By definition, +1 signifies pink erythema with four definite borders, +2 red erythema, +3 more intense erythema with palpable oedema, and +4 deep erythema with oedema and vesiculation. All the erythemas were graded conjointly by two experienced observers in the same room under the same lighting conditions. The subjects lay face down on a stretcher and the irradiated sites were inspected with a lamp composed of a 60 W incandescent bulb surrounded by a circular fluorescent tube.

Radiation exposures

Each subject was irradiated with one, two, or three, of the four different radiation sources. The minimal erythema dose (MED) and the minimal phototoxic dose (MPD) were first determined by using increments of exposures for each individual. The MED is defined as the minimum radiant exposure causing a +1 erythemal response at the time of the peak of erythema for a given spectral band. The MPD is defined as the minimum radiant exposure of UV-A causing a +1 erythemal response 48 h after UV exposure in a subject who has ingested methoxsalen (0·6 mg/kg) 2 h before the UV-A exposure. Multiples of the measured MED or MPD (3, 4, 5, 6 for UV-B; 20, 30, 50, 60 for UV-C; 1, 2, 3, 4 for UV-A; 1·5, 2, 2·5, 3 for PUVA) were then given to four 5 × 5 cm squares with the aim of subsequently selecting a site with a delayed erythema graded as +2 by our visual grading. Whenever several wavebands were used on one subject, an attempt was made to select squares which were graded at +2 and were of approximately equal erythema.

The times of maximal delayed erythema for UV-B (12–20 h), UV-C (5–9 h) and PUVA (48–72 h) are known and have been confirmed by previous experience in our laboratory. For UV-A the time for maximum erythema response was determined by irradiating separate volunteer subjects with the UV-A source used in this study and observing them every 2 h for 36 h after exposure. The UV-A induced erythema peaked at 8 h after exposure and this time was used for the visual grading in this study, although the grading was essentially the same at 24 h post irradiation. The multiples of MEDs (or MPDs) and the energy dose required to achieve +2 delayed erythema are listed in Table 1.

Biopsies

Using 1% local xylocaine anaesthesia and 4 mm Keyes punches, biopsies were performed in that 5 × 5 cm square, selected from the four exposed sites, which showed +2 erythema and was judged to be approximately equal to the erythema at biopsied sites exposed to other wavebands. For each waveband four biopsies were obtained at 24 h, 48 h, 72 h, and 7 days after exposure. The biopsies were performed near each corner of the square selected in order to minimize subsequent biopsies being influenced by inflammation induced from previous ones. A control biopsy was performed in an unexposed adjacent area on each volunteer. A total of seventy-three specimens were obtained including nine controls. The specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at a thickness of 4 μm, and stained with haematoxylin and eosin. The specimens were also stained with the periodic acid-Schiff technique.

The microscopic sections were then evaluated conjointly (through a double-headed microscope) in a

TABLE 1. Doses of UV used at various wavebands

Subject no./sex	UV-A (primarily 365 nm)		UV-B (290–320 nm)		UV-C (254 nm)		PUVA (320–400 nm)	
	No. of MED	Exposure dose (J/cm²)	No. of MED	Exposure dose (mJ/cm²) 280–320 nm	No. of MED	Exposure dose (mJ/cm²)	No. of MPD	Exposure dose (J/cm²)
1/F	2	100						
2/F	2	90					1·5	6
3/F	2	100					1·5	7
4/M			5	172	20	188		
5/M			3	248	50	354		
6/M			6	193	50	1475		
7/M			5	138	50	1475	3	8
8/M							2·5	9
9/M	2	90						

blinded fashion by two experienced dermatopathologists (Dr Mark and Dr Mihm) and the following histopathological changes were observed, segregated and quantified: dyskeratosis; spongiosis; enlargement of nucleoli of keratinocytes; quantity of dermal lymphocytic infiltrate; depth of dermal lymphocytic infiltrate; hypertrophy and hyperplasia of endothelial cells of capillaries and venules; nuclear dust around capillaries and venules; extravasation of red blood cells; hyperkeratosis; parakeratosis; increase or decrease of the granular layer; acanthosis; mitosis of keratinocytes; vacuolization of the cells of the Malpighian and basal layers; hypertrophy or hyperplasia of melanocytes; melanin pigment in the dermis; and dermal oedema. Dyskeratotic cells were taken to be those which had homogeneous and brightly eosinophilic cytoplasm and unrecognizable or pyknotic nuclei, and these were quantified on a scale of 0 (none), 1 (occasional), 2 (2–5 per high power field), 3 (6–10 per high power field), and 4 (more than 10 per high power field or a continuous band). All of the other changes were quantified on a scale of 0 (comparable to the control biopsy), 1 (minimal degree), 2 (moderate degree), and 3 (marked degree). Quantification was relative and done in such a manner that each of the three categories of positive quantity (+1, +2, +3) contained approximately one-third of the cases with positive findings.

Secondary investigation

In order to explore the limits within which the impact of reproducibility of our endpoint (approximately equal +2 delayed erythema at time of maximal delayed erythema) influenced our results additional volunteers were studied. Different degrees or shades of redness without palpable oedema (all within a +2 category by our grading system) were produced in several 2 × 2 cm squares at the belt line area of the back by using incremental exposure doses of the three different UV wavebands and PUVA. The increments used were 20% increases. At least three sites for each waveband were biopsied at 48 h and compared.

RESULTS

In UV-B and UV-C exposed sites dyskeratotic cells were scattered through the stratum Malpighii at 24 h and 48 h. By 72 h and 7 days they formed a continuous band in the upper stratum Malpighii or stratum corneum (Fig. 1). At all time intervals UV-A and PUVA caused fewer dyskeratotic cells which never coalesced into a band (Fig. 2). PUVA caused the least spongiosis at 24 and 48 h, but at 72 h and 7 days after exposure spongiosis was greatest in the PUVA-exposed sites (Fig. 3). Nucleoli of keratinocytes were normal 24 h after all exposures (Fig. 4). They increased in size and eosinophilia at 48 and 72 h after UV-B and UV-C exposure and returned to normal by 7 days. After UV-A and PUVA exposure nucleoli were enlarged only in the biopsies taken at 7 days after exposure.

UV-A elicited the greatest degree of inflammation at 24, 48, and 72 h after exposure (Figs 5 and 6), in terms of both quantity and depth of cell infiltrate. At 7 days the greatest infiltrate was observed in the biopsies from PUVA-treated patients. The quantity and depth of the infiltrate were combined into a single value because the two changes were found to be closely related in the following manner: a minimal infiltrate (Grade 1) generally involved only the papillary dermis, a moderate infiltrate (Grade 2) involved the upper third of the reticular dermis, and a marked infiltrate (Grade 3) involved the middle third and rarely the lower third of the reticular dermis. Endothelial cell swelling, nuclear dust, and extravasation of red blood cells were generally observed together. These dermal findings became more pronounced at 72 h and 7 days with PUVA and 72 h with UV-A. Lesser degrees were observed with UV-B and UV-C (Fig. 7).

Some histological findings varied with the time interval after exposure but not with each different waveband, and some findings did not correlate with either the time interval or waveband. Within the

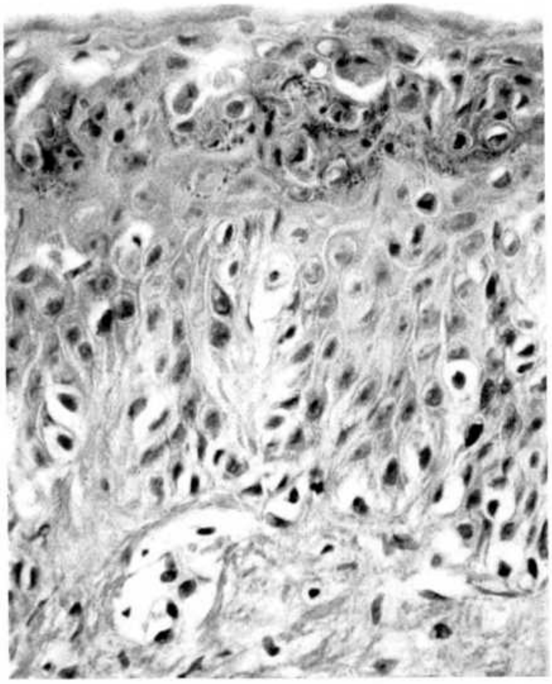

FIGURE 1. Epidermal changes observed 72 h after exposure to UV-C producing a +2 delayed erythema. An almost continuous band of dyskeratotic cells is present in the upper stratum Malpighii and in the stratum granulosum (H & E, × 400).

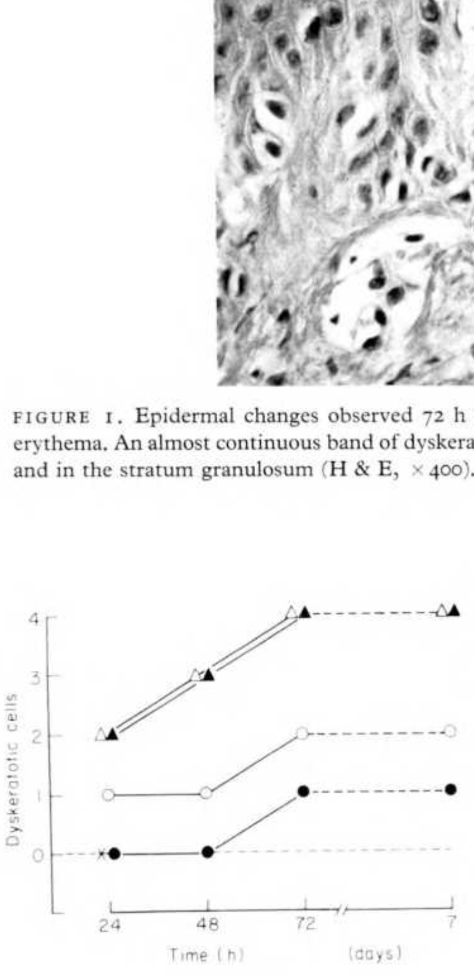

Fig.2.

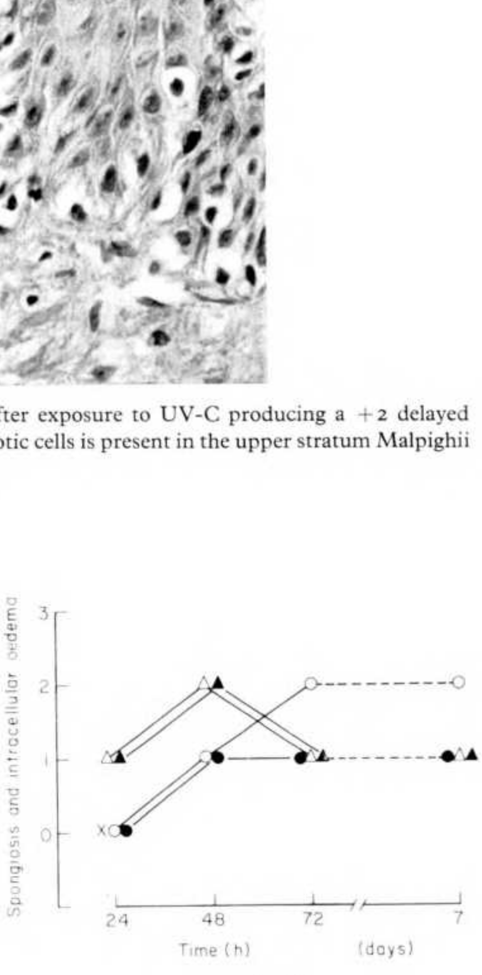

Fig.3.

FIGURE 2. Dyskeratotic cells versus time after exposure to different ultraviolet wavebands. (o) None observed; (1) occasionally present but not in all high-power fields; (2) 2–5 per high power field; (3) 6–10 per high power field; (4) more than 10 per high-power field or continuous band of dyskeratotic cells in the upper stratum Malpighii or stratum corneum.

FIGURE 3. Spongiosis versus time after exposure to different ultraviolet wavebands. (o) Control; (1) minimal; (2) moderate to severe; (3) microvesicles. (○) PUVA; (●) UV-A; (△) UV-B; (▲) UV-C; (×) control.

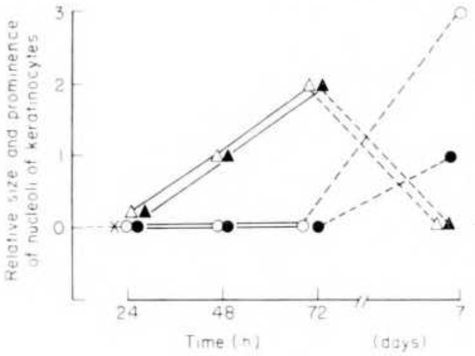

Fig.4.

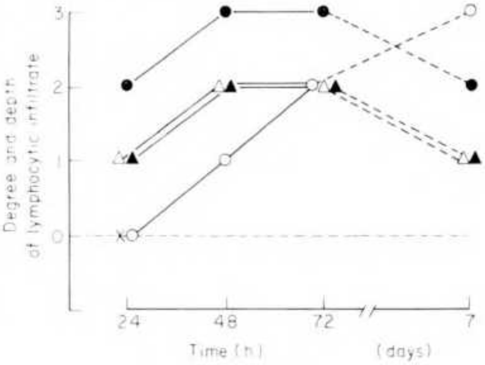

Fig.5.

FIGURE 4. Size of nucleoli of keratinocytes versus time after exposure to different ultraviolet wavebands. (0) Control; (1) minimal enlargement; (2) moderate enlargement; (3) marked enlargement.

FIGURE 5. Quantity of lymphocytic infiltrate versus time after exposure to different ultraviolet wavebands. (0) Control; (1) minimal; (2) moderate; (3) marked. (○) PUVA; (●) UV-A; (△) UV-B; (▲) UV-C; (×) control.

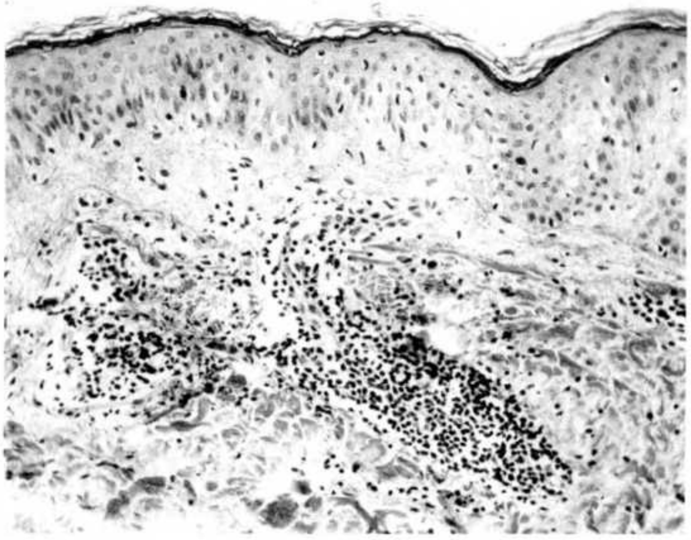

FIGURE 6. Dermal inflammatory infiltrate observed 72 h after exposure to UV-A producing a +2 delayed erythema. This Grade 3 infiltrate is composed primarily of lymphocytes, arranged in both a perivascular and diffuse distribution, and extending as deep as the middle third of the reticular dermis. Endothelial swelling and scant nuclear dust are also present (H & E, × 25).

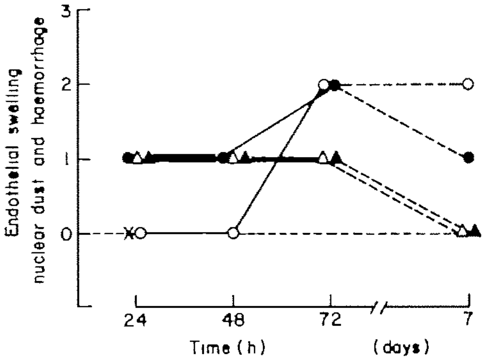

FIGURE 7. Endothelial swelling, nuclear dust, and haemorrhage versus time after exposure to different ultraviolet wavebands. (0) Control; (1) Minimal; (2) Moderate; (3) Marked. (○) PUVA; (●) UV-A; (△) UV-B; (▲) UV-C; (×) Control.

epidermis, hyperkeratosis, parakeratosis and acanthosis appeared concurrently. They were absent for all wavebands at 24 and 48 h and appeared in sites irradiated with UV-B and UV-C at 72 h and in PUVA-treated individuals at 7 days. They never appeared in sites irradiated with UV-A and were most striking after UV-C. The granular layer was focally absent with UV-B and UV-C at 24 h and 48 h, and focally increased with all wavebands at 72 h and 7 days. Mitotic figures in keratinocytes were observed infrequently. Melanocytes were not increased in number over the time course of this study by light microscopic examination of haematoxylin-eosin sections nor was melanin pigment consistently increased in quantity with exposure to any of the spectral bands studied.

Intracellular oedema of moderate degree was noted with all wavebands at all time intervals. Most vacuolated keratinocytes caused by exposure to UV-A, UV-B, or UV-C stained negatively by the periodic acid-Schiff stain both before and after diastase, indicating that the cytoplasmic vacuoles did not contain glycogen or mucopolysaccharides. At 48 h and 72 h after PUVA exposure a few vacuolated keratinocytes stained positively for glycogen. Vacuolization of the basal layer of moderate degree was noted at all time intervals and wavelengths.

Oedema of the papillary dermis and perivenular space was slightly increased with UV-A and PUVA at 72 h and 7 days but was not observed with any wavebands at 24 h or 48 h.

Secondary investigation

The histological changes present 48 h after ultraviolet exposure doses producing different degrees of erythema (all within our 2+ visual grading) were identical for any given waveband.

DISCUSSION

Cutaneous histopathology produced by ultraviolet radiation has been described by several authors (Keller, 1924; Uhlmann, 1931; Miescher, 1957; Daniels, Brophy & Lobitz, 1961; Nix, 1965). Microscopic observations of individual cases have been reported after psoralen ingestion and subsequent ultraviolet exposure (Becker, 1959; Zimmerman, 1959). Although the effects of ultraviolet radiation on the histological appearance of the skin was at times described in detail, early investigators lacked the technical equipment necessary for precise production and measurement of UV wavebands used.

More recent studies have attempted to compare the histology of different UV portions of the spectrum. Stern (1972) compared the histological effects of UV-B, UV-C, and UV-A and concluded that in all instances the common denominator was vascular injury. He used suberythemogenic doses of UV-A and unequal erythemogenic doses of UV-B and UV-C and biopsied only at 24 h. Willis & Cylus (1977) compared histology of sites irradiated with UV-A and UV-B without stating the degree of erythema and biopsied only at 48 h. They did not observe dyskeratosis with UV-A exposure. In a subsequent report of the ultrastructural changes induced by UV-A, dyskeratotic cells were noted (Kumakiri, Hashimoto & Willis, 1977). In the current study dyskeratotic cells, thought by some to be specific for 'sunburn' (Johnson, Mandell & Daniels, 1972), were observed after UV-A exposure but were few in number and not seen until 72 h or 7 days. Although various mechanisms have been postulated to explain the production of dyskeratotic cells by UV radiation (Wilgram *et al.*, 1970; Johnson *et al.*, 1972; Kerr, Wyllie & Currie, 1972; Olson & Everett, 1975; Woodcock & Magnus, 1976) the exact pathophysiology remains unclear. This study shows that the appearance and number of these 'sunburn' cells is clearly dependent on the wavelength of irradiation and on the time interval after exposure.

In this study the basis for comparison of histological effects is approximately equal delayed erythema. In our secondary investigation the histological changes for the slightly different degrees of +2 erythema were identical for any given waveband. These findings indicate that, over the range of our +2 visual grading system, histological changes for each wavelength region are constant.

Our findings show that ultraviolet induced histological changes in skin vary with time after exposure and with waveband of irradiation. Of the time intervals used here, maximum histological changes of both the epidermis and dermis were present at 48 to 72 h for UV-A, UV-B and UV-C, while for PUVA maximal changes were seen at 7 days. The time required for the skin to complete its histological response to a single ultraviolet exposure was least with UV-B and greatest with PUVA. For UV-A and PUVA the time for completion is unknown, since the histological changes were still obvious at 7 days. This observation must be considered when studying chronic or cumulative histological changes induced by repeated exposures to PUVA or UV-A because specimens obtained at one week or less after an exposure will still exhibit histological sequelae of that last exposure. In general, the shorter UV wavebands, UV-C and UV-B, affected the epidermis to a greater degree than the longer wavebands. The longer waveband, UV-A, affected the dermis to a greater degree at all times.

This study does not conclusively support any of the theories of UV erythema production (epidermal vs. dermal; direct vs. mediated damage). The prominent dermal changes produced by UV-A and PUVA compared to those by UV-C and UV-B may be related to greater penetration of longer wavelengths into the skin (Everett *et al.*, 1966). This study may be useful to future investigators in evaluating and interpreting histological changes produced by UV radiation. The knowledge of a detailed description of the sequential histological changes following ultraviolet exposure in normal skin is essential before one can identify abnormal histological responses to electromagnetic radiation.

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Immunofluorescence studies on eosinophilic granulocytes

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SUMMARY

Immunofluorescence techniques were used to find out whether immunoglobulins (Ig) and complement (C3) determinants are present on the membrane of eosinophilic granulocytes in atopic patients and in patients with an allergic contact dermatitis. Normal healthy individuals served as controls. The studies were performed with eosinophilic granulocytes in suspension and in thin cell layers.

The demonstration of the presence of cytoplasmic Ig and C3 in eosinophilic granulocytes was carried out in skin specimens taken 20–30 min after injection of the antigen and in specimens taken from positive patch tests 48 h after application of the antigen.

No immunoglobulins or C3 determinants could be demonstrated on the membrane of eosinophilic granulocytes in the patients or the healthy controls, when the suspension methods were used. However, in thin cell layers and in skin sections the eosinophilic granulocytes showed a positive fluorescence of the cytoplasm after having been exposed to various conjugates and a FITC solution.

It is concluded that the commonly used immunofluorescence techniques tend to give non-specific staining results, most likely due to reactivity of the FITC marker of the conjugates with basic proteins present in the cytoplasm of the eosinophilic granulocyte.

The B-lymphoid cells and neutrophilic granulocytes, isolated from the peripheral blood of atopic patients and patients with allergic contact dermatitis, show different patterns of membrane determinants (Cormane, Husz & Hamerlinck, 1974; Carpeto, Winkelmann & Jordon, 1976; Braathen *et al.*, 1978). In atopic dermatitis a predominance of IgE bearing cells was found whereas in allergic contact dermatitis an increase of IgD bearing cells was noted.

Since neutrophilic granulocytes and eosinophilic granulocytes share some properties, and since Ig and C3 have been demonstrated on the membrane of neutrophilic granulocytes in healthy controls and in patients with dermatitis, a study was undertaken to investigate whether these determinants can also be detected on the membranes or in the cytoplasm of eosinophilic granulocytes in atopic patients and in patients with allergic contact dermatitis by means of defined immunofluorescence methods.

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MATERIALS AND METHODS

Eosinophils were obtained from five patients with atopic dermatitis, five patients with allergic contact dermatitis and from five normal healthy department members as controls.

Punch biopsies were taken from the positive skin reactions to atopic allergens 20–30 min after the injection of the antigen, and from the positive patch test 48 h after application of the antigen. The various antigens to which the two groups of patients were sensitive are listed in Tables 1 and 2. Punch biopsies taken from the contralateral sites of the uninvolved skin were used as controls.

TABLE 1. Patients with contact dermatitis

Patient no.	Positive patch tests after 48 h
1	Tincture of iodide 0·5%
2	Potassium dichromate 0·5% in vaseline
3	Nickel sulphate 2·5% in vaseline
4	Balsam of Peru 25% in vaseline
5	Formaldehyde 0·5% in vaseline

TABLE 2. Patients with atopic dermatitis

Patient no.	Positive intracutaneous reaction after 20–30 min allergens*
1	Pollen 100 NE/ml
2	Human dander 0·1%
3	Human dander 0·1%
4	Mixed animal epidermal products 0·01%
5	Human dander 0·1%

* NV Haarlems Allergenen Laboratorium, Haarlem, The Netherlands.

Chemicals and reagents. For separation of the eosinophilic granulocytes the same chemicals and reagents were used as described earlier (Berretty & Cormane, 1978). Sodium dodecyl sulphate (SDS) (Sigma Chemical Company, St Louis, U.S.A.) was diluted to 0·025% (final concentration) in borate buffer (pH 8·4, ionic strength 0·1).

The characteristics of FTC-protein conjugates are given in Table 3.

The methods for the separation of the eosinophilic granulocytes that were adopted in this study have been described in detail elsewhere (Berretty & Cormane, 1978).

Immunofluorescence studies. Direct immunofluorescence (DIF) was performed on isolated eosinophilic granulocytes from the peripheral blood in suspension as well as in thin cell layers and skin sections. FITC solution (Table 3) was used as a control.

Suspension method. Direct staining of the eosinophilic granulocytes was carried out by suspending 0·1 ml washed eosinophils (1×10^5) in 0·1 ml of the conjugate (final dilution of the conjugates was

1:40). This mixture was then incubated at 4°C for 30 min. After incubation the cells were washed twice for 5 min at 200 *g* in isotonic NaCl and resuspended in 0·2 ml isotonic NaCl solution.

Immunofluorescence examination was carried out in suspension for the five main classes of immunoglobulins, the third component of complement (C3) and F(ab). Possible positive cells were determined by examining the microscopic field alternately in blue narrow-band (480–490 nm) epiillumination for fluorescence and in conventional light. The eosinophilic granulocytes are easily distinguished from neutrophilic granulocytes by their typical granules. Tryphan blue staining for viability of the cell preparations was performed before IF staining and was generally more than 95%.

Cell layer preparation. For the cell layer technique the same method was adapted in this study as described in detail earlier (Cormane *et al.*, 1974).

Preparation of skin biopsies. The skin specimens were prepared following the techniques described earlier (Cormane, Szabo & Hauge, 1970). The immunofluorescence studies of the skin sections have

TABLE 3. Characteristics of the FTC-protein conjugates

Conjugates	Batch no.	F/P ratio (g/mg)	Antibody unitage	Working unitage approx.
Rabbit anti-human* IgG/FTC	KH 16-113-A1	1·51	4	0·066
Rabbit anti-human* IgA/FTC	KH 14-13-A02	1·76	2	0·033
Rabbit anti-human* IgM/FTC	KH 15-10-A2	1·93	8	0·133
Rabbit anti-human* IgD/FTC	KH 20-05-A02	2·03	2	0·033
Rabbit anti-human* IgE/FTC	KH 25-04-A01	1·63	—	—
Rabbit anti-human* C3c/FTC	KH 41-9-P01	2·85	2	0·033
Rabbit anti-swine† IgG/FTC	2-969	1·17	—	—
Goat anti-rabbit‡ IgG/FTC	7-274	—	—	—
FITC solution				2 μg/ml

* The unconjugated antisera were obtained from the Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam, and were labelled in our Laboratory by S.S. Ashgar, Ph.D.

† Nordic Immunological Laboratories, Tilburg, The Netherlands.

‡ Contain antibodies directed against heavy and light chains of rabbit IgG.

— Not studied.

FTC content of conjugated was determined by the method of McKinney, Pearce & Spillane (1964) using fluorescein diacetate (FDA) as reference standard. Protein content was measured by the method of Lowry *et al.* (1951). Antibody unitage conjugates was determined in a standard gel diffusion test using 1 mg/ml respective antigens except in case of FTC/C3c conjugate where normal serum was used as a source of antigen, against doubling dilutions of conjugates as described by Beutner, Sepulveda & Barnett (1968). A 1 mg/ml solution of human IgG, IgM, IgA and IgD contained 6·6, 129, 30·5 and 100 antigen units respectively in relation to international reference serum. Staining properties and specificity of the class-specific conjugates were tested in IF in normal and monoclonal human bone marrow plasmacytes.

been carried out in comparison with the aniline-blue staining according to the method of Eder (Eder, 1960; Berretty & Cormane, 1978).

Microscopy. In this study the same light and fluorescence microscope equipment was used as described elsewhere (Hunyadi, Hamerlinck & Cormane, 1976).

RESULTS

Eosinophilic granulocytes in suspension failed to show surface immunoglobulins or complement (C3), irrespective of whether they were obtained from the controls or the patients. However, all the eosinophilic granulocytes of the healthy controls, the atopic patients and the patients with allergic contact dermatitis used for this study showed a positive fluorescence staining in the cell layer technique with all the conjugates and the FITC solution (Table 3).

In accordance with the latter mentioned findings were the observations obtained with the DIF method of the biopsies taken from a positive patch test. All the conjugates stained the eosinophilic granulocytes very conspicuously, mostly granular, sometimes more or less homogeneous.

Comparable results were obtained in the skin section taken from a positive skin reaction to an atopic allergen. In this case the eosinophilic granulocytes were present mainly in the lumen of the dermal vessels (Fig. 1).

It is of note that neither in the uninvolved skin of the patients with allergic contact dermatitis, nor in the uninvolved skin of the atopic patients, could positive fluorescent eosinophilic granulocytes be demonstrated with the DIF method.

Control conjugates. After having been exposed to the conjugates (GAR/FTC and RAS/FTC) used for the specificity tests, all the eosinophilic granulocytes in the thin cell layers and in the skin sections showed a bright fluorescence. There appeared to be no difference between the staining results of the monospecific homologous conjugates and the heterologous conjugates.

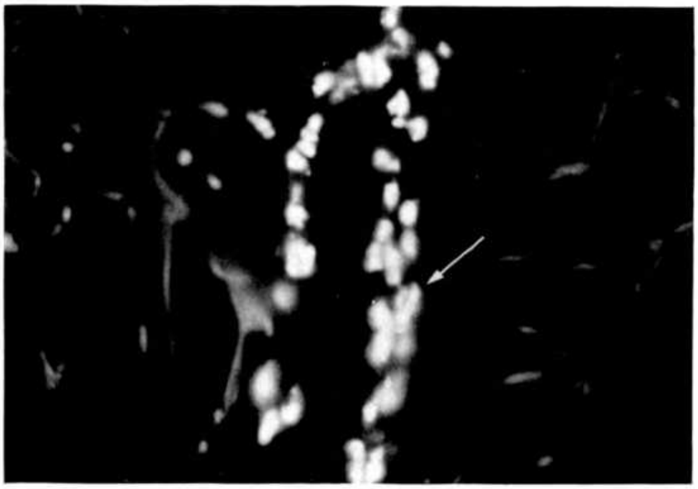

FIGURE 1. Cryostat section of a biopsy taken from a positive skin reaction after an intracutaneous injection with an atopic allergen. Note the fluorescent cytoplasm of the eosinophilic granulocytes in an intradermal vessel. DIF: RAHuIgM/FTC (Blue narrow-band epi-illumination, × 400).

Blocking effect of sodium dodecyl sulphate (*SDS*). When the cell layers or skin sections were incubated in 0·025% SDS before the usual immunofluorescence techniques were performed, a remarkable reduction of fluorescence was seen. Instead of the bright fluorescence only a weak yellow-brown fluorescence could be observed. This blocking phenomenon could be demonstrated in the cell layers as well as in the sections, with all the conjugates used. No difference between the eosinophilic granulocytes of the healthy controls or those of the patients could be found.

DISCUSSION

With the suspension methods no surface receptors belonging to the five main immunoglobulin classes and complement (C3) could be seen on the membrane of eosinophilic granulocytes in patients or in healthy controls, using fluorescence techniques. These observations confirm the findings of Gupta *et al.* (1976) who could not demonstrate surface immunoglobulins by means of immunofluorescence techniques. However, these results are not in accordance with the results we obtained in the cell layer methods and in the skin sections. All the homologous as well as the heterologous conjugates and the FITC solution gave positive staining results.

With regard to the IF methods currently used we have to keep in mind that the staining results depend upon the physicochemical properties of the conjugates used, the conditions of the substrates and the microscopic techniques (Gianetti & Cormane, 1971).

It has been shown that the eosinophilic granulocytes ingest immune complexes (Litt, 1964). However, the fact that we were unable to demonstrate Ig- and C-determinants on the membrane of the eosinophilic granulocytes, the most likely protein fragment in the cytoplasm of these cells, suggests a rapid pinocytosis after complex formation with the conjugates. However, incubation with the conjugates was performed at 4°C. The addition of sodium azide failed to show any cap formation. These conditions are well known to delay pinocytosis processes.

One may wonder why monospecific heterologous conjugates also gave positive staining results which cast doubt on the reliability of these fluorescence results. This phenomenon could be explained by cross-reactivity and by a physicochemical adsorption of the conjugates to the substrates that is not based on an immune reaction.

As our results suggest that complex factors influence the fluorescence in eosinophilic granulocytes, we tried to determine the origin of these phenomena by the performance of several blocking tests. Recently Iszui, Lambert & Miescher (1976) postulated that cationic proteins can disturb the immune complex forming by the formation of different types of complexes. The granules of eosinophilic granulocytes contain high concentrations of basic protein (Olsson *et al.*, 1977). Granular arginine-rich cationic proteins, called the major basic proteins, are responsible for the staining results obtained with acid dyes as eosin and aniline-blue. As FITC is also an acid dye similar staining results were obtained.

Based on the resemblance of the IF staining results and those obtained with acid dyes, it is tempting to assume that the major basic proteins of the granules of the eosinophils induced the non-specific fluorescence. Therefore we performed comparable blocking reactions as did Iszui *et al.* (1976).

Since sodium dodecyl sulphate is a denaturant, it might dissociate certain proteins attached to the eosinophilic structure of the cytoplasm. The proteins that have been dissociated might be basic proteins. The basic protein of the eosinophilic granulocytes might attach itself to the conjugate just as C1q can attach itself to IgG and IgM. In any case the fluorescein marker of the conjugates do stain eosinophilic granulocytes.

It is concluded that the major basic proteins may be one of the keys to the role of eosinophilic granulocytes. However, these proteins interfere unfavourably with the immunofluorescence staining results of eosinophilic granulocytes in cell layers and skin sections.

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Activation of complement—a mechanism for the inflammation in acne

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SUMMARY

An immunofluorescence investigation of thirty-six non-inflamed and thirty-four inflamed acne lesions has been carried out. Fluorescence for C3, unaccompanied by fluorescence for immunoglobulins, was found in the walls of dermal blood vessels or at the basement membrane zone of the comedo or at both these sites in fifty-seven lesions. Fluorescence for C3 accompanied by fluorescence for immunoglobulins was found in nine lesions. In two papules and three nodules the comedo was surrounded or replaced by a mononuclear cell infiltrate. These findings have been taken to indicate that cellular immune mechanisms are a late event and activation of complement is an early event in the pathogenesis of inflammation in acne. The mechanism of complement activation remains uncertain.

The physical signs in acne that draw the attention of the patient to his spots are predominantly the inflamed lesions. Evidence suggests, however, that it is certain of the non-inflamed lesions which are the originators of the inflamed lesions (Kligman, 1974). Under androgenic control the sebaceous glands increase in size at puberty and patients with acne produce more sebum than control subjects (Cunliffe & Shuster, 1969). This phenomenon affects most of the pilosebaceous units, but at any one time only a small number are involved in the development of the clinical lesions. The lesions clinically consist of non-inflamed lesions (comedones)—the whiteheads and blackheads; and the inflamed lesions—the papules, pustules and nodules. Whiteheads and blackheads represent the accumulation of a mixture of keratin-rich protein, lipids and bacteria within the pilosebaceous duct.

It is not known what is the sequence of events responsible for the evolution of a non-inflamed into an inflamed lesion. A few years ago free fatty acids derived from sebaceous triglycerides by bacterial lipases were considered important but support for this concept is now declining (Strauss & Pochi, 1965; Puhvel & Sakamoto, 1977). There is evidence that patients with acne develop immunity to *P. acnes*. High levels of complement fixing antibodies to *P. acnes* occur in patients with severe acne while lower levels occur in patients with mild acne or without acne (Puhvel, Hoffman & Sternberg, 1966). Cellular sensitivity to *P. acnes* is detectable in subjects with a long history of severe acne

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(Gowland *et al.*, 1978). We have further considered the possibility that immune mechanisms might be involved in the pathogenesis of acne lesions, by applying the techniques of immunofluorescence to the study of acne biopsies.

MATERIALS AND METHODS

Thirty-six non-inflamed and thirty-four inflamed acne lesions were obtained by biopsy from the backs of sixty-four patients. The non-inflamed lesions comprised six early whitehead lesions, fifteen developed whiteheads and fifteen blackheads; the inflamed lesions were ten early papules, seventeen developed papules and seven nodules.

All biopsies included uninvolved pilosebaceous units so that each biopsy would serve as its own control. During the period of study we examined involved and uninvolved skin from patients with a variety of skin diseases.

The method and reagents used have been described previously (Scott, Scott & Losowsky, 1977). In brief, biopsies were quick frozen, using a bench mounted CO_2 freezing unit. Sections cut from the block in a cryostat were washed in phosphate buffered saline, fixed in 95% ethyl alcohol. Sections were then reacted in direct staining experiments with fluorescein isothiocyanate (FITC) labelled gammaglobulin fractions of monovalent antiserum against human IgG, IgA, IgM, C1q and C3. An FITC labelled anti-fibrinogen antiserum was used for the detection of fibrin related antigens in sections. The indirect staining procedure was used for the detection of IgE.

RESULTS

Tables 1 and 2, which summarize the results of the immunofluorescence studies, indicate that, when present, fluorescence was seen usually in the walls of the blood vessels or in the basement membrane

TABLE 1. Immunofluorescence findings in thirty-six non-inflamed acne lesions

	Immunofluorescence pattern		
Lesion	Blood vessels	B.M. zone of lesion	Number with fluorescence pattern
Early whitehead (N = 6)	None	None	3/6
	None	C3	1/6
	C3	C3	2/6
Blackhead (N = 15)	None	None	2/15
	C3	None	4/15
	C3	C3	2/15
	C3, F*	None	3/15
	C3, F	C1q, F	2/15
	M†, C1q, C3, F	C3	2/15
Developed Whitehead (N = 15)	None	None	1/15
	None	C3	8/15
	C3	C1q, F	2/15
	C3	C3	3/15
	M, C1q, C3, F	C3	1/15

* F = Fibrin related antigens.

† M = IGM.

zone of the involved pilosebaceous unit or at both these sites. Tables 1 and 2 show that in 6/36 comedones (three early whiteheads, two blackheads and one whitehead), and in 5/34 inflamed lesions (two papules and three nodules) there was no fluorescence at these sites whatsoever. No basement membrane zone fluorescence was found in uninvolved pilosebaceous units in any acne biopsy.

In eighteen non-inflamed and twenty-one inflamed lesions granular or granular-cum-homogeneous fluorescence for C3, in the absence of any fluorescence for immunoglobulins, was found in the walls of dermal blood vessels both near the lesions and at a distance from them.

C3 was accompanied by fibrin related antigens in five non-inflamed and sixteen inflamed lesions.

We saw no evidence of IgA or IgE deposits; and granular fluorescence for immunoglobulins in the walls of dermal blood vessels was a relatively uncommon finding. It was encountered in nine lesions, three non-inflammatory and six inflammatory.

Examination of the basement membrane of the comedones showed homogeneous, linear fluorescence for C3 in 19/36 of the non-inflamed lesions and 16/34 inflamed lesions. The basement membrane zone fluorescence for C3 was sometimes found as a broad irregular band around the comedo and was accompanied by fluorescence for fibrin related antigens in seven inflamed lesions.

Linearly distributed granular deposits of IgG, IgM and C3 were found in the basement membrane zone of a comedo in one early inflamed lesion and in one papule. Seven lesions, four non-inflamed and three inflamed, showed basement membrane zone fluorescence for C1q and fibrin related antigens in the absence of fluorescence for immunoglobulins or C3.

TABLE 2. Immunofluorescence findings in thirty-four inflamed acne lesions

Immunofluorescence pattern			
Lesion	Blood vessels	B.M. zone comedo	Number with fluorescence pattern
Early Papule (N = 10)	None	C3	2/10
	C3	C3	2/10
	C3, F*	None	4/10
	C3, F	C3, F	1/10
	G, M, C3	G, M, C3	1/10
Papule (N = 16)	None	—	2/17
	C3	C3	1/17
	C3, F	None	2/17
	C3, F	C3, F	6/17
	C3, F	C1q, F	3/17
	M†, C1q, C3, F	F	2/17
	G, M, C3	G, M, C3	1/17
Nodule (N = 8)	None	—	3/7
	C3	C3	2/7
	G, M, C1q, C3	—	1/7
	M, C1q, C3	—	1/7

— comedo not recognized. Lesion consisted of a heavy mononuclear cell infiltrate replacing collagen and elastic autofluorescence in the dermis.

* F = Fibrin related antigens.

† M = IGM.

No trace of a comedo was seen in two papules and five nodules. In these cases the lesion consisted of a mononuclear cell infiltrate and in only two out of these seven lesions was there evidence of deposition of immunoglobulins and complement in the blood vessels walls.

In six papules, the pleomorphic cellular infiltrate included plasmacytoid cells showing fluorescence for IgM and cells showing cytoplasmic fluorescence for C3.

DISCUSSION

Tables 1 and 2 show that three patterns of immunofluorescence were found in the acne lesions:

(1) Fluorescence for C3 with or without fluorescence for fibrin related antigens in the absence of fluorescence for immunoglobulins or C1q.

(2) Fluorescence for immunoglobulins together with fluorescence for C1q and/or fluorescence for C3 with or without fluorescence for fibrin related antigens.

(3) Fluorescence for fibrin related antigens or for fibrin related antigens and C1q in the absence of fluorescence for immunoglobulins and C3.

These patterns and their distribution in the lesions are set out in Table 3.

The interpretation of the finding of fluorescence for C1q and fibrin in the absence of fluorescence for immunoglobulins and C3 at the basement membrane zone of the comedo in seven lesions is uncertain. It may reflect local activation of C1q by plasmin.

The findings of granular deposits of immunoglobulins and complement in tissues has been taken as suggestive evidence for the deposition or *in situ* formation of immune complexes (Wilson, 1976; Parish, 1970). We found such evidence in 3/36 non-inflamed lesions and 6/64 inflamed lesions. That evidence of immune complex formation was a relatively infrequent finding suggests that the deposition or formation of immune complexes is not responsible for the initiation of the inflammation in acne.

Similarly our observation that mononuclear cell infiltrates surrounding or replacing the comedo were present in papules but not in whiteheads or blackheads suggests that cellular immune mechanisms come into play only late in the acne lesion. These findings are in agreement with those of Gowland *et al.* (1978) who found a high degree of cellular immunity only in a proportion of patients with severe acne.

Sites showing fluorescence for C3 but not for immunoglobulins or C1q were found in forty-eight lesions. Findings of this nature, e.g. Dahl & McGibbon (1976), might denote alternative pathway activation of complement, and we would regard such findings as strongly suggestive of such a mode of activation. However the failure to detect immunoglobulins and C1q does not establish their absence. It is conceivable that these immunoreactants were present, but in concentrations too low to be detected by our techniques. The early lesions of acne vulgaris are of the non-inflamed type, i.e. whiteheads and blackheads; it is the former which is said to have the greatest potential for developing into an inflamed lesion (Kligman, 1974).

It will be seen from Table 3 that C3 was found as frequently in blackheads as it was found in whiteheads. This is perhaps surprising. However, it suggests that blackheads may have more inflammatory potential than they are usually credited with.

It might be suggested that the detection of C3 in tissues has nothing to do with the inflammatory process. The possibility that the detection of C3 in lesions merely reflects the activity of proteases during the interval between biopsy freezing was examined in a previous study (Scott *et al.*, 1977). In that study twelve sets of jejunal biopsies were taken—each set consisting of two biopsies taken from the one patient with dermatitis herpetiformis on one occasion. One biopsy was frozen immediately

TABLE 3. Distribution of immunofluorescent pattern in seventy acne lesions

		Early whitehead (N = 6)	Blackhead (N = 15)	Developed whitehead (N = 15)	Early papule (N = 10)	Papule (N = 17)	Nodule (N = 7)
$C_3 \pm F$	B.M. zone comedo only	1	0	8	0	0	0
	B.M. zone comedo + blood vessels	2	2	3	3	7	2
	Blood vessels only	0	9	2	4	5	0
$Ig's + C_3 \pm C1q + F^*$	B.M. zone comedo only	0	0	0	0	0	0
	B.M. zone comedo + blood vessels	0	0	0	1	1	0
	Blood vessels only	0	2	1	0	2	2
$F \pm C1q$	B.M. zone comedo	0	2	2	0	3	0
No fluorescence	Mononuclear cell infiltrate surrounding	0	0	0	0	2	3
No fluorescence	No mononuclear cell infiltrate	3	2	1	0	0	0

* F = Fibrin related antigens.

and one after a 30 min delay. C3 was found no less frequently in biopsies frozen immediately than in those frozen after a delay. It seems unlikely therefore that in the present study the detection of C3 reflects the action of tissue proteases.

Our evidence in non-inflamed lesions which could be interpreted as the stimulation of the alternative pathway activation of complement suggests that this event might be responsible for the conversion of a non-inflamed lesion to an inflamed lesion. This activation generates products such as C3a and C5a which, together with the possible release of other inflammatory mediators, will increase vascular permeability with the subsequent leakage of plasma to produce oedema. C3a and C5a will also attract polymorphonuclear leukocytes, and so help to explain the histology of a developing inflamed acne lesion. There is also evidence that *P. acnes* will activate *in vitro* the alternative pathway by cleaving C3 in normal human serum. *P. acnes* is not unique in stimulating the alternative pathway; *Actinomyces viscosus* produces similar events in periodontal disease (Allison, Schorlemmer & Bitter-Suermann, 1976) and the gonococcus may produce skin lesions by stimulating the alternative pathway in benign gonococcal sepsis (Scherer & Braun-Falco, 1976).

Although our findings are open to a variety of interpretations, the fact that C3, only occasionally accompanied by immunoglobulins, was detected in fifty-seven lesions suggests that activation of C3 (perhaps via alternative pathway) plays a part in the pathogenesis of the inflammatory acne lesion. It may well be true that the alternative complement pathway plays a major role in the activation of C3 as suggested by Massey, Mowbray & Noble (1978), but formal immunohistological evidence of the action of the alternative pathway awaits the use of antisera to factor B.

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Pharmacology and Treatment

A double-blind trial of a zinc sulphate/citrate complex and tetracycline in the treatment of acne vulgaris

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SUMMARY

Forty-eight patients participated in a 3-month double-blind study to compare the effect of orally administered zinc sulphate/citrate complex and tetracycline hydrochloride in acne vulgaris. Tetracycline significantly reduced the overall grade, and the number of non-inflamed lesions, papules and pustules by the third month. It also reduced significantly the non-inflamed lesions and papules at the end of the second month. In contrast, zinc therapy only had a significant effect on the pustules at the third month.

The results indicate that tetracycline is far superior to the zinc complex in patients with moderately severe acne.

Antimicrobial preparations, taken systemically or applied topically, have been the cornerstone of treatment for acne vulgaris over the past 20 years (Cunliffe & Cotterill, 1975). Although understanding of the hormonal control of the sebaceous gland has increased recently (Shuster & Thody, 1974) this advance has not produced a satisfactory alternative approach to acne treatment.

Considerable interest has therefore been generated by Swedish reports (Michaëlsson, Vahlquist & Juhlin, 1977c; Hillstrom *et al.*, 1977) that zinc taken orally is as effective as tetracycline in acne. This was reflected by a recent leading article in the *British Medical Journal* (1977).

We therefore decided to attempt confirmation of these findings in a double-blind trial comparing zinc and tetracycline orally. The design of the trial was somewhat different from that of the Swedish studies, in that 250 mg of tetracycline twice daily was used in this study, compared with only 250 mg/day in the Swedish reports. The larger dose is commonly used for the treatment of acne in Britain.

MATERIALS AND METHODS

The double-blind trial was performed during the months from October 1977 to March 1978 to avoid the modifying influence of ultraviolet radiation. Patients received no treatment for a minimum period

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of 4 weeks before the trial commenced. No treatment, other than tetracycline (250 mg twice daily taken half-an-hour before food and not with milk) or a zinc sulphate/citrate complex (1 three times daily), was given during the trial.

The patients attended on five visits. The initial two control visits were within 48 h of each other. At the second visit treatment was allocated and subsequent follow-up was at monthly intervals.

Clinical assessment was conducted as follows:

(*a*) *Assessment of overall grade of acne severity*. This was determined by looking at the patient's face and giving a numerical grade, which in this trial ranged from 0 (no acne) to 6 (severe acne). This is a scoring system which had been in use in the department for several years. Originally it was adopted from the method of Burton *et al.* (1971) and the scoring scale has been enlarged. Detailed studies have indicated the reproducibility of the method (Cunliffe & Gould, unpublished observations).

(*b*) *Counting the total numbers of:* blackheads and whiteheads, papules, small pustules, deep pustules and nodules (referred to as large inflamed lesions).

It should be noted that assessment of lesions is often not as simple as it may appear because spots sometimes show features of two types of lesion. For example a resolving nodule may be the size of a papule but still quite large. As long as the assessor is constant in his judgement of the lesions then spot counting is reproducible (Cunliffe & Gould, unpublished observations).

(*c*) *Patients' assessment*. The patients were asked to judge for themselves whether any improvement or deterioration had occurred at the third, fourth and fifth visit. This was achieved using a 20 cm visual analogue scale.

After the control visits the patients were put into pairs with respect to their overall grade and number of inflamed lesions. Individual patients were then randomly allocated either to the zinc group or to the tetracycline group.

RESULTS

A total of forty-eight patients commenced the trial, twenty-four in each group; of these twenty in each group completed therapy. The four defaulters on tetracycline did so for no stated reason.

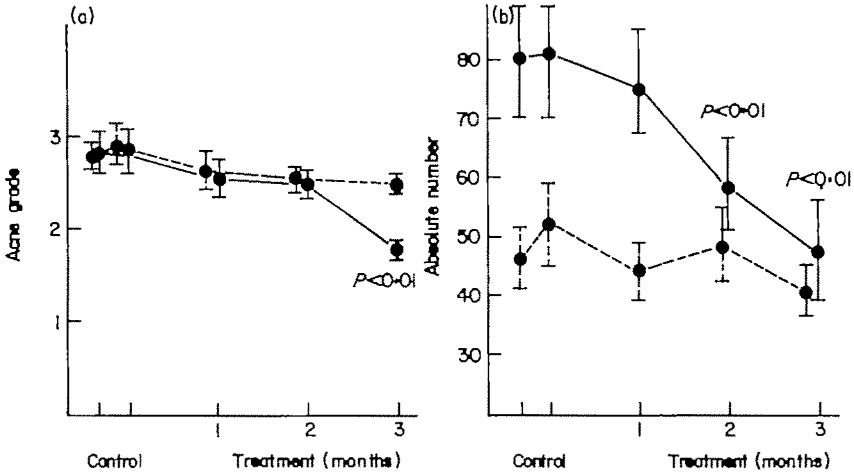

FIGURE 1

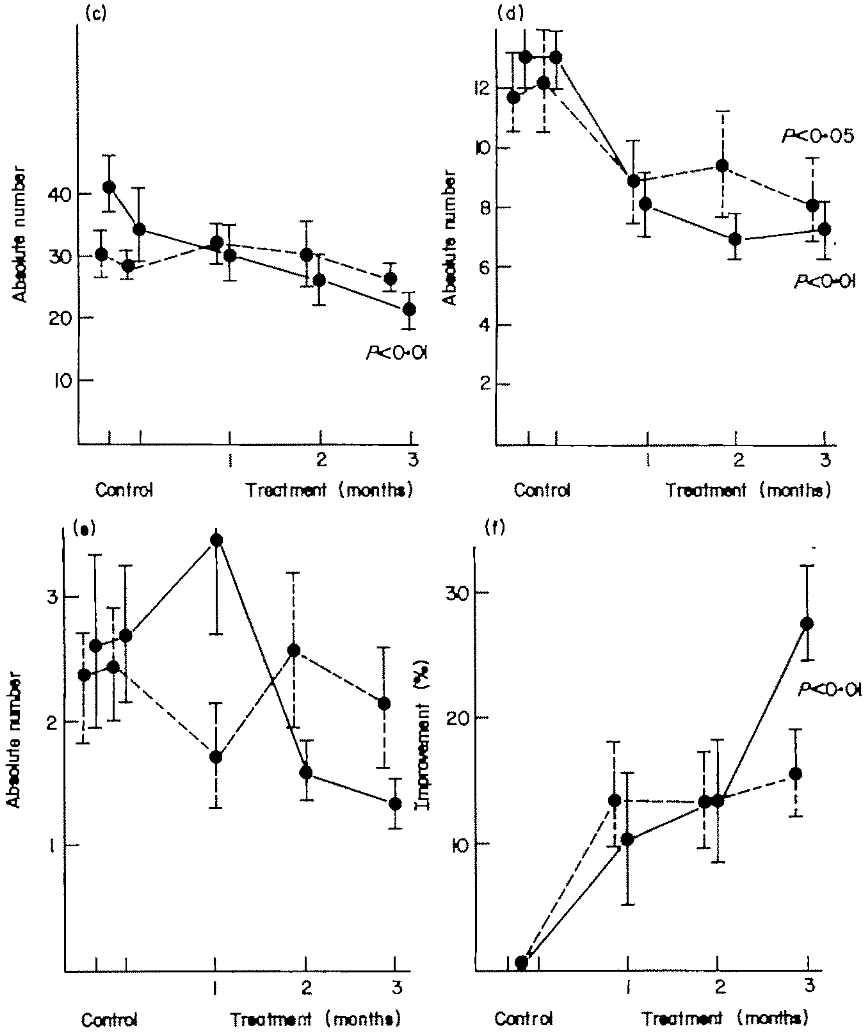

FIGURE 1. Effects of tetracycline and zinc therapies (mean ± s.d.). (Solid line) tetracycline, N = 20; (broken line) zinc, N = 20. (a) Overall grade; (b) blackheads and whiteheads (non-inflamed lesions); (c) papules; (d) small pustules; (e) nodules and deep pustules; (f) patient assessment.

Two patients treated with zinc left because of deterioration in their acne, and two because of nausea and abdominal pain.

The results (expressed as means and standard errors) are shown in Fig. 1a–f. The statistical significance of these findings was determined by Wilcoxon's matched pairs signed ranks test (Armitage, 1971). This non-parametric test was chosen because (a) most of the data are not normally distributed and (b) data for acne grade have rank but no clearly defined interval.

From these results tetracycline produces a significant improvement in overall grade at the end of the third month of treatment and the papule count was decreased at the end of the second and third months. Small pustules were also significantly decreased by the end of the third month and blackheads and whiteheads showed a significant decrease after the second and third months. The only significant objective change produced by zinc was in the reduction in pustules after 3 months treatment. Significant subjective improvement was recorded in the tetracycline group at the third visit.

DISCUSSION

There have been three reports (Michaëlsson *et al.*, 1977c; Hillstrom *et al.*, 1977; Michaëlsson, Juhlin & Ljunghall, 1977a) indicating the beneficial effect of zinc in acne. Our study however has failed to confirm this and we are supported by one other Scandinavian report (Weisman, Wadskov & Søndergaard, 1977) in which the authors could not distinguish any difference between the effects of oral zinc and placebo.

Zinc may act by stabilizing lysosomes (Chvapil, 1973), or by limiting the rate of neutrophil chemotaxis and complement activation (Weston *et al.*, 1977), essential for the inflammatory response in acne. Our studies detected clinical improvement only in pustules in the zinc treated group, and this would fit with the latter observations (Weston *et al.*, 1977).

It could be argued that although initially the two groups were comparable as far as overall grade and inflamed lesions were concerned, there were important differences in the number of non-inflamed lesions. There was indeed a significant difference between the two groups initially in the non-inflamed lesions and it may be that a higher initial number of lesions favours a better response. These data merit comment. In the study we attempted to match the patients as accurately as possible, using as the predominant factor the inflamed lesions and overall grade. There was nothing that could be done to improve the matching of the non-inflamed lesions. However, as the data are logarithmically distributed, the apparent difference (between eighty and forty lesions) is greater than the real difference. The zinc treated group had a mean of forty non-inflamed lesions, and we have shown in other studies that tetracycline (Cunliffe *et al.*, 1973) as well as other preparations such as benzoyl peroxide (Cunliffe, Dodman & Ead, 1978) can significantly reduce this number of non-inflamed lesions.

There are two explanations for the difference between those studies which showed zinc to be effective, and our own observations. Firstly, a larger dose of tetracycline was used in our trial and this is the dose most widely prescribed for acne therapy in the U.K. However, this observation alone does not explain the relative lack of response to zinc. Secondly, the Swedish group (Cunliffe, Juhlin & Michaëlsson, personal communication) has confirmed that they used patients who, on average, had a greater severity of acne than those in this trial. Our results support the suggestion that if zinc has any effect it may be in those patients with many pustules.

The zinc used in this study was the same zinc (zinc sulphate/citrate complex) as that used by the Swedish authors. It is much less toxic than the zinc sulphate used in the U.K. We have shown in an uncontrolled trial that zinc sulphate alone (manufactured in the U.K.) produces a considerable incidence of gastro-intestinal upsets (either nausea, vomiting or diarrhoea) and in one patient perforation of an asymptomatic gastric ulcer (Cunliffe, 1979). Even so, two patients failed to complete the present trial owing to unacceptable side-effects of zinc therapy.

What is the future for oral zinc in the treatment of acne vulgaris ? We need to compare zinc sulphate in a double-blind trial with tetracycline in patients with very severe pustular acne, but such therapy should only be given in a controlled study. Tetracycline, in our experience, remains superior to zinc sulphate complex in patients with moderate acne and zinc sulphate/citrate complex should not be used in the treatment of such patients.

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Levamisole therapy for multiple warts

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SUMMARY

Levamisole, a wide-spectrum antiparasitic drug, increases cellular immunity *in vivo* and *in vitro*. Patients with verruca vulgaris may have defective cell mediated immune mechanisms.

Levamisole was given to twenty-two patients with multiple warts, 5 mg/kg body weight on 3 consecutive days every fortnight.

Patients' lymphocytes were studied for E-rosette formation before therapy. E-rosette counts were lower in patients compared with controls and a significant increase was obtained after *in vitro* incubation with levamisole.

Seventeen patients were cured in 1–4 months, there were four failures and one patient showed marked improvement.

It is concluded that patients with multiple warts have defective cell-mediated mechanisms as far as the E-rosette count is concerned and that levamisole increases *in vitro* E-rosette formation and is useful in the treatment of these patients.

Levamisole (-(-)-2,3,5,6-tetrahydro-6-phenylimidazo (2,2-b) thiazole hydrochloride), a wide-spectrum antiparasitic drug, has been shown to increase cellular immunity, both *in vitro* and *in vivo* (Ramot *et al.*, 1976).

Renoux *et al.* (1972) reported that levamisole had the ability to cure or reduce metastases from an experimentally induced tumour in mice. In anergic patients with cancer, levamisole restored cutaneous delayed hypersensitivity to dinitrochlorobenzene and P.P.D. (Tripodi, Parks & Brugmans, 1973).

A great deal of data has been accumulated in the past few years showing the immunopotentiating effect of levamisole, and at the present time the drug appears to be useful, as shown by double-blind study, in rheumatoid arthritis, aphthous stomatitis and recurrent respiratory infection in children. It is also of some use in other conditions such as herpes simplex infections (Symoens & Rosenthal, 1977). In patients with resectable lung cancer, it appears to change favourably the course of the disease (Swierenga, Gooszen & Vanderschueren, 1975).

In vitro, levamisole appears to increase the number of E-rosette forming cells (thymus-derived) in patients suffering from Hodgkin's disease (Biniaminov & Ramot, 1975) and also in other malignancies (Moncada-Gonzalez, Rodriguez-Escobedo & Castanedo de Alba, 1976).

Patients with warts have defective cell mediated mechanisms as measured by *in vitro* response to

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PHA and PPD (Morison, 1975a). It is believed that for the resolution of the lesions immune mechanisms are necessary (Morison, 1975b; Pyrhönen & Johanssone, 1975).

Helin & Berg (1974) gave levamisole to ten children with warts and observed regression of lesions in nine of them; no *in vitro* studies were reported in that series.

PATIENTS AND METHODS

Twenty-two patients with multiple verruca vulgaris were treated with levamisole*, 5 mg/kg body weight on 3 consecutive days every 2 weeks. Most of patients received twelve cycles of therapy. They ranged in age from 5 to 33 years (mean 15 years); thirteen were male and nine female. They had between ten and several dozen lesions most of them located on hands, forearms and face. There were four cases with warts in the oral cavity. Patients were healthy otherwise.

Patients had had the lesions for more than 6 months, in some cases up to 7 years. They were resistant to conventional forms of therapy. Before treatment was started, heparinized blood was obtained in eighteen of the twenty-two patients and a Ficoll-Hypaque gradient performed (Böyum, 1968). E-rosette counts were done after a short and a long incubation period to assess both active and total E rosettes (Wybran & Fudenberg, 1973); both populations of cells seemed to represent T lymphocytes.

A parallel set of tests was done and incubated at 37°C with 40 μg/ml of levamisole, and E rosettes counted after a period of 30 min (Biniaminov & Ramot, 1975). Similar studies were done in blood obtained from a group of normal individuals of similar age.

RESULTS

Patients with multiple verrucae had lower E-rosette counts, both active and totals, and when analysed by Student's 't'-test the difference from normal controls was statistically significant ($P<0{\cdot}01$) (Table I).

TABLE 1. E-rosette forming cells (mean ± s.d.) in normal individuals and in eighteen patients with multiple verruca vulgaris

	E-rosette forming cells (%)	
Group	Active	Total
Controls	28 ± 2	66 ± 4
Patients	22 ± 4	51 ± 8
	$P<0{\cdot}01$	$P<0{\cdot}01$

Incubation of patients' lymphocytes with levamisole increased E-rosette counts, active as well as totals, from baseline levels (Table 2). Paired t-test analysis demonstrated a significant difference ($P<0{\cdot}001$) (Dixon & Massey, 1969).

Among the twenty-two patients treated with levamisole, four failures were observed, that is patients who after completing twelve cycles of therapy showed no change or just a very mild improvement in their lesions. Seventeen (77%) were cured in a period of time ranging from 1 to 4 months. Lesions disappeared without producing symptoms, except for mild pruritus in a few cases, and they left no scars.

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* Supplied as 50 mg tablets by Johnson and Johnson de México.

TABLE 2. Effect of levamisole *in vitro* on E-rosette forming cells (mean ± s.d.) in eighteen patients with multiple verruca vulgaris

	Before levamisole	After levamisole	*P*
Active E rosettes	22 ± 4	36·5 ± 7	< 0·001
Total E rosettes	51 ± 8	61 ± 5	< 0·001

A 27-year-old woman with numerous periungual warts, present for 7 years, showed marked improvement at the end of the 24-week observation period but since there was not complete resolution of the lesions this case is not included with the cured group (Table 3).

The longest period of observation has been 18 months after therapy was completed and there have been no recurrences. No side effects were noted other than mild gastro-intestinal irritation in three patients.

TABLE 3. *In vivo* effect of levamisole in patients with multiple verruca vulgaris (twenty-two cases)

Result	Number	%
Cured	17	77
Failed	4	18
Improved	1	4

DISCUSSION

These results clearly show that levamisole acts as an *in vitro* immunostimulator in patients with multiple warts. This condition known to be produced by a viral agent affects people at random and usually has a good prognosis, lesions disappearing after a short period of time, spontaneously or under appropriate standard treatment.

The host's response against the infection depends on intact immune mechanisms, particularly those involving cell-mediated immunity (Tagami *et al.*, 1974). This study shows that at least by one parameter, that is, the E-rosette count, the above mentioned mechanism is faulty in verruca patients.

Levamisole appears to influence the course of the disease as was shown in this series. Several mechanisms of action have been proposed for the drug acting as an immunopotentiating agent. It may have a thymosin-like effect. It also appears to act as a weak mitogen or as an adjuvant of known mitogens such as Concanavalin A (Merluzzi *et al.*, 1975; Pabst & Crawford, 1975). Levamisole also raises cyclic GMP by inhibiting cyclic GMP-phosphodiesterase in lymphocytes thereby increasing such lymphocyte functions as proliferation, lymphokine production and lymphocytotoxicity (Hadden *et al.*, 1975). In order to explain the immunostimulating effect of levamisole, it could be very important to consider its effect on macrophage proliferation and phagocytosis which are increased by the drug (Hadden *et al.*, 1975).

We believe that, in the light of the facts presented here, levamisole can be considered as useful in the treatment of patients suffering from warts—not to all of them, but to those affected by numerous lesions, present for long periods of time, resistant to conventional forms of therapy, and particularly if one or more parameters of cell-mediated immunity are shown to be defective. Controversial reports in the literature regarding the use of levamisole in verruca patients (Helin *et al.*, 1974; Symoens & Rosenthal, 1977) may be due to different criteria applied in the selection of patients, and to different periods of therapy.

ACKNOWLEDGMENT

Acknowledgment is given to Ricardo Garza, PhD who reviewed the manuscript and performed the statistical analysis of the data.

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Case Reports

Hereditary hypotrichosis

A PREVIOUSLY UNDESCRIBED SYNDROME

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SUMMARY

A syndrome of hereditary hypotrichosis with an unusual natural history and clinical features was encountered in a Caucasian family of four generations with a total of twenty-four members of whom eleven were affected. The mode of inheritance was autosomal dominant with variable penetrance. The loss of hair involved the scalp, eyebrows, eyelashes and body hair, manifesting itself in the school years and progressing to almost complete baldness. There were no associated abnormalities and no sex limitation. Clinical, genetic, biochemical, mechanical, histological and immunological aspects were studied. Essential differences between this type of hereditary hypotrichosis and others previously recorded are stressed.

Hereditary hypotrichosis is rare but distinct varieties with different clinical features and modes of inheritance have been described. Some involve hair alone or are in association with ectodermal abnormalities while others show features of skeletal, chromosomal or amino acid defects. A completely unfamiliar type of hypotrichosis was encountered in a Caucasian family, originally of British stock but domiciled for many generations in South Africa. A search of the literature revealed no record of an exactly similar pattern.

CASE REPORTS

The first three cases seen were as follows:

Case 1. Mrs E.B., aged 48, a Caucasian female whose hair had commenced to thin and disappear at the age of 7 years, having been completely normal since birth. Her scalp hair had diminished gradually leaving a few wispy hairs. Eyelashes and eyebrows had disappeared and body hair became sparse (Fig. 1).

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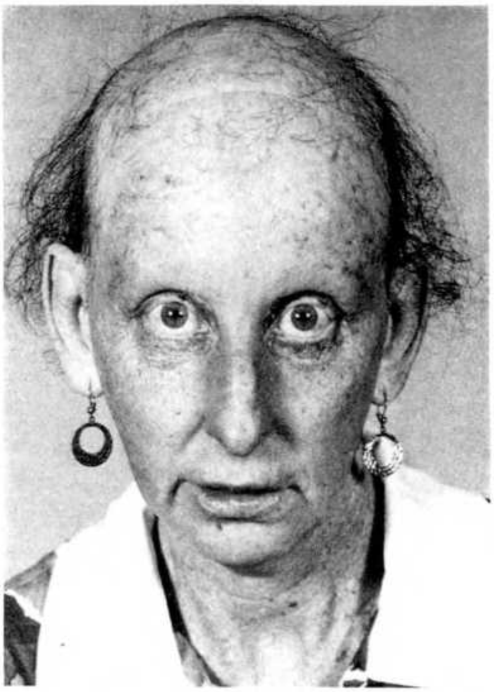


FIGURE 1. Hereditary hypotrichosis. Case 1, aged 48 years.

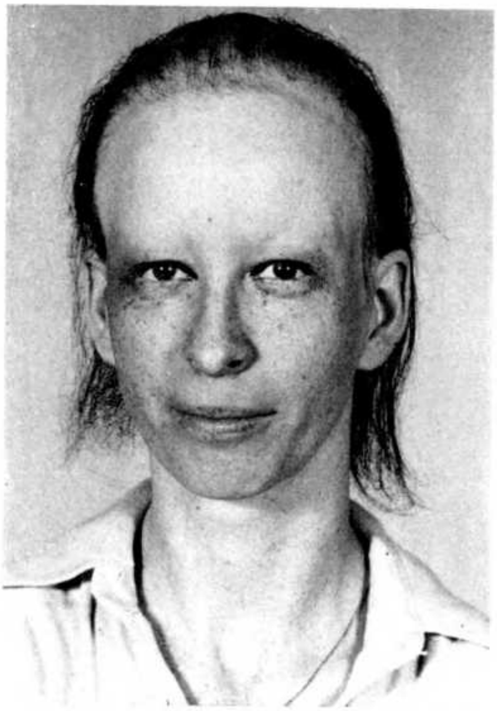


FIGURE 2. Hereditary hypotrichosis. Case 2, aged 28 years.

Case 2. Miss L.B., aged 28, elder daughter of Mrs E.B. had a similar pattern of hypotrichosis with normal hair until the age of 8 years when scalp, facial and body hair had disappeared gradually (Fig. 2).

Case 3. Miss V.B., aged 22, the younger daughter of Mrs E.B., had hypotrichosis of exactly the same pattern as that of her mother and sister (Fig. 3).

Mr N.B., aged 21, the son of Mrs E.B. is the fourth member of the family. His hair pattern has been completely normal since birth with no evidence of hypotrichosis.

Investigations

With unstinted help from members of the family a pedigree was prepared (Fig. 4) and of twenty-four members in four generations eleven were found to be affected. The earliest member of the family to be traced was Mr J.B. (I, 1) who had arrived in South Africa from London in 1904 accompanied by his wife and daughter (II, 1). According to the family Mr J.B., now deceased, had always worn a wig and had no eyelashes or eyebrows. All available descendents, consisting of four children, seven grand-children and twelve great-grand-children were examined. Those unable to be seen were sent questionnaires, asked to record defects of hair, skin, nails, teeth, eyes, colour and texture of hair and also the presence of any congenital abnormalities (mental, anatomical, etc.). Samples of hairs were requested and the majority of the family complied. Details were analysed and hairs were subjected to various investigative procedures.

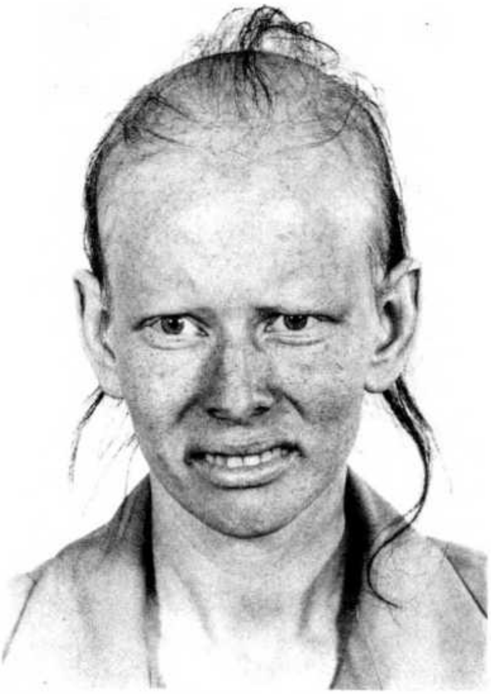


FIGURE 3. Hereditary hypotrichosis. Case 3, aged 22 years.

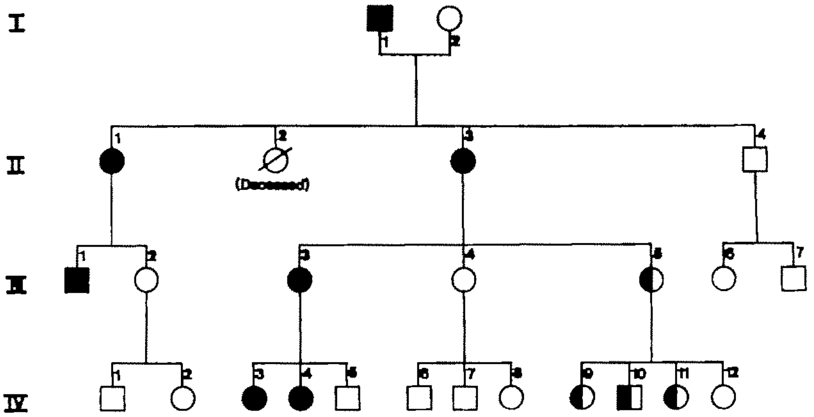


FIGURE 4. Family pedigree. (○) female unaffected; (□) male unaffected; (●) female affected (wears wig); (■) male affected (wears wig); (◐) female partially affected (no wig); (◧) male partially affected (no wig).

RESULTS

Clinical features

The history was the same in all cases, male or female, with variation only in the severity of the hypotrichosis. An arbitrary division was made into severe (those wearing wigs) and partial (those not wearing wigs) for purposes of the pedigree chart.

It was found that:

(1) all children had normal hair until school age, then slow and diffuse hair loss commenced affecting scalp, eyebrows, eyelashes and leaving only wispy hairs on the scalp. Body hair was slow to develop and invariably sparse;

(2) terminal stage was reached by the age of 20–30 years and there was no change in hair colour;

(3) the scalp looked and felt thin and atrophic;

(4) no keratosis pilaris was observed;

(5) facial complexions were pallid;

(6) there was no associated abnormality of nails, teeth, eyes or other systems;

(7) psychological and emotional states were surprisingly normal considering the cosmetic disabilities of these severely affected females;

(8) the partially affected individuals did not wear wigs and merely accepted their 'poor quality' hair. Their main concern was whether their children would inherit the trait as they were well aware that no treatment was available for this condition.

Genetic aspects

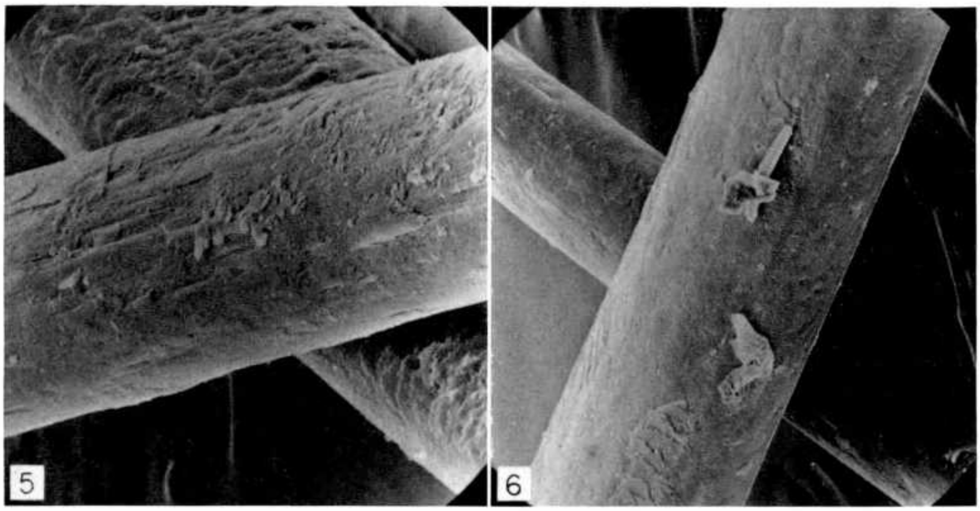
The pedigree (Fig. 4) provided the following information: autosomal dominant transmission; approximately 50% of the offspring affected; no sex limitation; every generation affected, severely or partially; no transmission from unaffected parents; no consanguinity recorded; no twins in the family; no record of two affected individuals marrying.

There is now a fifth generation of three infants, all too young to be assessed as the condition appears only in later years.

Microscopic studies

Light microscopy. Appearances under the light microscope merely confirmed the clinical finding of 'fine quality' hairs with reduced diameters of the shafts. Apart from this there were no detectable changes.

Scanning electron microscopy. Hairs from the three most severely affected subjects showed a mixture of normal and abnormal cuticular patterns. The changes varied from mild to severe 'weathering' with partial or complete absence of overlapping cuticular scales and some longitudinal fissuring. This suggested that the cuticle was almost completely worn away and that longitudinal cortical cells were beginning to show through (Figs 5 and 6). No hairs showed any evidence of the characteristic 'brush-ends' of trichorrhexis nodosa.



FIGURES 5 AND 6. S.E.M. cuticular changes. (5) moderate (× 550); (6) severe (× 500).

Histology

Minor changes only were seen in the scalp biopsies of Mrs E.B. and her two daughters. In all cases the epidermis was thin and atrophic with some dilated follicles. Hair shafts were present in many of the follicles while others contained granular basophilic material. A perifollicular lymphocytic infiltration was present in some areas. The sebaceous glands were abundant and normally developed.

Immunology

Direct immunofluorescence staining of scalp biopsy sections was performed using mono-specific fluorescein-conjugated antisera to IgA, IgM, fibrin and to C1q, C3, C4 components of complement. No immunofluorescence was demonstrated.

Biochemistry

In all three cases thin layer chromatography for amino acids, using ninhydrin visualizing agent, showed a normal pattern.

It was not possible to estimate the cysteine content of hairs as there were no facilities available for this investigation.

Chromosomal studies

Both daughters (IV, 3 and IV, 4) of Mrs E.B. (III, 3) have a satellited No. 17 chromosome (17PS +) but the significance of this is obscure. It has been considered to be a variant (marker chromosome) in normal individuals and because neither their mother nor their father has this pattern it is probably not linked to the hair disorder. Several other members of the family, partially affected (III, 5 and IV, 9) and one unaffected (IV, 12), exhibited normal karyotypes.

Dermoglyphics

The palms of Mrs E.B. and her two daughters have very hypoplastic ridge development, ridge dissociation and no palmar patterns (Fig. 7). This suggests that there might be some connection between poor volar skin and hair loss. Dermoglyphics of three of the partially affected individuals IV, 9, IV, 10 and IV, 11 were unremarkable.

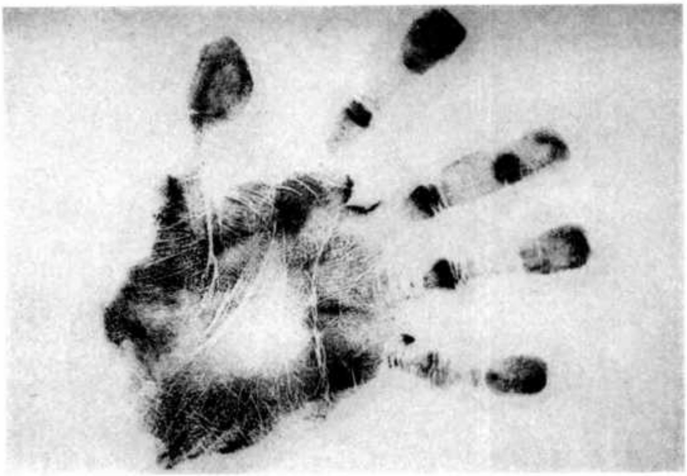


FIGURE 7. Palm dermoglyphic. Case 3.

DISCUSSION

Genetic hair disorders may be classified into recessive, dominant, X-linked, sporadic and unknown types of inheritance. These disorders may be associated with abnormalities of amino acid metabolism, with chromosomal, ectodermal or skeletal defects or they may be limited to the hair alone (Table 1). Some familiar examples of hypotrichosis with associated abnormalities are shown in Table 2.

When the disorder is limited to the hair alone there may be obvious structural defects, e.g. monilethrix, pseudomonilethrix, pili torti, bamboo, invaginated, wooly or kinky hairs, etc. while others are without apparent defects. Many families belonging to this latter group have been recorded, including those described by Lederman (1896), Petersen (1915), Jeanselme & Rime (1924), Pajtas (1950), Toribio & Quiñones (1974). The families bearing the closest resemblance to the one under discussion are those of Jeanselme & Rime and Toribio & Quiñones. Similarities and differences are shown in Table 3.

Jeanselme & Rime (1924) described a French family where the onset of hypotrichosis started at 8–9 months with scalp, facial and body hair involved. The scalp was thickened with some twisted hairs and keratosis pilaris present. Inheritance was autosomal dominant involving thirty-five indi-

TABLE 1. Classification of genetic hair disorders

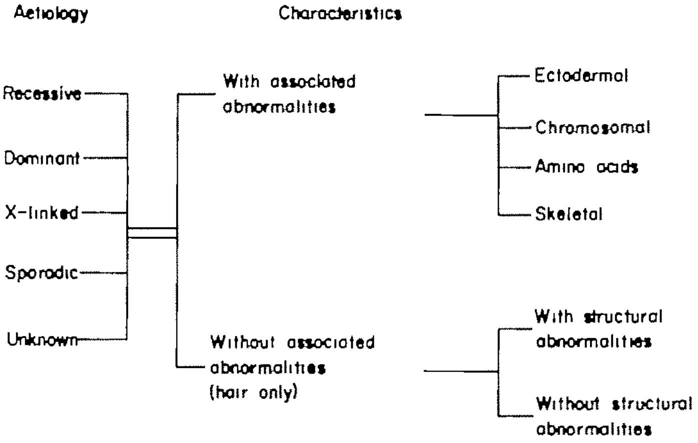

Aetiology
Characteristics
Recessive
Dominant
X-linked
Sporadic
Unknown
With associated abnormalities
Ectodermal
Chromosomal
Amino acids
Skeletal
Without associated abnormalities (hair only)
With structural abnormalities
Without structural abnormalities

TABLE 2. Examples of genetically determined hypotrichosis

Disorder	Inheritance	Phenotype	Extent of involvement
Anhidrotic ectodermal dysplasia	X-linked	Hypotrichosis	Epidermis, teeth, sweat glands, nails
Hypotrichosis, syndactyly, retinitis	Autosomal recessive	Hypotrichosis	Syndactyly, retinitis pigmentosa
Hallerman Strief syndrome	Autosomal recessive	Hypotrichosis	Bird-like facies, dwarfism, microphthalmos, cataracts
Marie Unna type	Autosomal dominant	Hypotrichosis	Keratosis pilaris, mental retardation, scarring alopecia, folliculitis

viduals with fourteen affected members in five generations. The percentage of affected subjects fell from 100% in generation I to 18·7% in generation IV.

Toribio & Quiñones (1974) reported a large Spanish family with onset of hypotrichosis at 5–12 years, limited entirely to the scalp which was atrophic. No abnormalities of hair structure were present nor was there any evidence of keratosis pilaris. The inheritance was autosomal dominant involving 213 members in eight generations and twenty-eight affected individuals. No partial forms were seen and the average percentage affected in each generation was 50%.

In the present family involvement of scalp, facial and body hair commenced at 7–13 years, with atrophy of the scalp and no evidence of keratosis pilaris or hair structure abnormality. There are twenty-four members in four generations and eleven affected individuals. Partial forms are present and the average number involved in each generation is approximately 50%.

TABLE 3. Clinical, genetic and investigative similarities and differences between three families

Hypotrichosis—hair only involved

	Jeanselme & Rime (1924)	Toribio & Quinones (1974)	Bentley-Phillips & Grace (1979)
Scalp hair	Absent	Absent	Absent
Eyebrows	Absent	Present	Absent
Eyelashes	Absent	Present	Absent
Body hair	Absent	Present	Absent
Age of onset	8–9 months	5–12 years	7–13 years
Keratosis pilaris	Present	Nil	Nil
Scalp skin	Thickened, leathery	Thin	Thin and atrophic
Colour of face	Milky with red blotches	Pallid	Pallid
Residual hair colour	Not recorded	Unchanged	Unchanged
Partial forms	Not recorded	None	Some
Nails	Fragile	Normal	Normal
Genetics			
Transmission	Autosomal dominant	Autosomal dominant	Autotosomal dominant
Sex limitation	None	None	None
% affected	Reducing in each generation	Approximately 50%	Approximately 50%
Every generation	Affected	Affected	Affected
Transmission from unaffected	None	None	None
Marriage between two affected	Not recorded	Not recorded	Not recorded
Consanguinity	Not recorded	Not recorded	Not recorded
Chromosomes		Normal	Abnormal ?
Dermoglyphics		Normal	Abnormal
Microscopy			
Light	Twisted spiral hairs	Normal	Normal
S.E.M.	Not available	Normal (early stage) Abnormal (late stage)	Abnormal appearances
Biopsy	Normal	Normal	Minor alterations
Immunology	Not available	Not recorded	Negative
Amino acids	Not available	Normal	Normal
Tensile strength	Not recorded	Not recorded	Results awaited
Trichorrhexis nodosa	Not recorded	None	None

TABLE 4. Noteworthy differences between the present family and the nearest approach (Jeanselme & Rime, 1924)

	Jeanselme & Rime (1924)	Bentley-Phillips & Grace (1979)
Age of onset	8 months	5–13 years
Spiral twisted hairs	Present	Absent
Keratosis pilaris	Present	Absent
Incidence	Reducing each generation	50% throughout
Biopsy of scalp	Normal	Abnormal
Scalp skin	Leathery and thickened	Thin and atrophic
Nails	Fragile	Normal

The most noteworthy differences between this family and that of the one bearing the closest resemblance (Jeanselme & Rime, 1924) are summarized in Table 4.

The aetiology of hereditary hypotrichosis is obscure but Toribio & Quiñones (1974) had the opportunity of admitting eight of their patients to hospital for exhaustive investigations which included total and differential counts of hair follicles on serial section. They concluded that the follicles gradually reduced the duration of their cycles. The duration of anagen became so short that the hairs did not grow long enough to show through the follicular pores. This also occurs in the vellus follicles of the forehead and nose. Their conclusion appears to be a valid assumption and would explain the peculiarities of this type of hereditary hypotrichosis.

A search of the literature has failed to reveal a similar pattern and it is therefore considered that this is a previously undescribed variant of hereditary hypotrichosis.

ACKNOWLEDGMENTS

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British Journal of Dermatology (1979) **101,** 341.

Focal epithelial hyperplasia of the oral mucosa

A CASE REPORT FROM THE UNITED KINGDOM

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SUMMARY

A case of focal epithelial hyperplasia of the oral mucosa (Heck's disease) is presented, which appears to be the first to be described in the United Kingdom. Virus particles were found in tissue from the lesions studied by electron microscopy, and identified as belonging to the papilloma virus sub-group of the family of papovaviruses.

Since 1956 there have been several reports of a condition consisting of multiple discrete papules of the oral mucosa occurring in American Indians from Central and South America. Archard, Heck & Stanley (1965) made the first complete description of this entity in a study of nineteen children, the majority of whom were Navajo Indians; details were given of the characteristic histological appearance of the lesions, and the term focal epithelial hyperplasia was introduced (Heck's disease). The condition most commonly occurs in the native populations of under-developed countries, and more than 600 cases have subsequently been recorded, mainly in the dental literature. Although other factors may be of relevance, previous reports have suggested a viral aetiology. We describe a case of focal epithelial hyperplasia in the United Kingdom in which papovavirus particles belonging to the sub-group papilloma virus were identified by electron microscopy.

CASE REPORT

The patient was a 26-year-old male West Indian steel-worker born in Trinidad. He had lived in the United Kingdom for 20 years and had not travelled abroad during this time. He gave a recent history of multiple small asymptomatic lesions inside the mouth which appeared initially on the lower lip, and spread over several months to the upper lip, the sides of the tongue and the inner aspect of the cheeks. There had been no ulceration of the oral mucosa. There was no past history of warts, and the patient did not know of any other person with similar oral lesions.

Examination

Numerous symmetrical soft white papules, 2–4 mm in diameter, were present on the buccal mucosa

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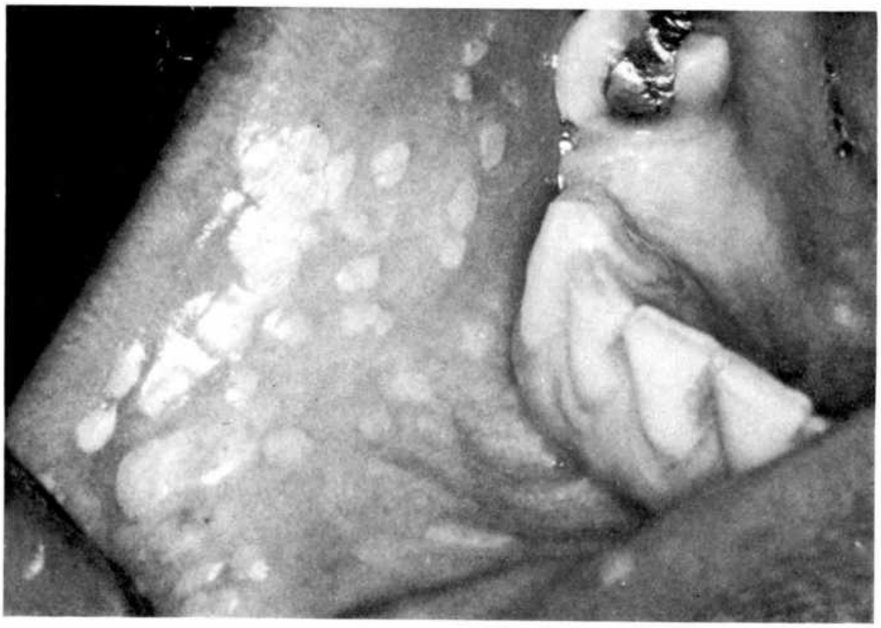

FIGURE 1. Oral lesions of focal epithelial hyperplasia.

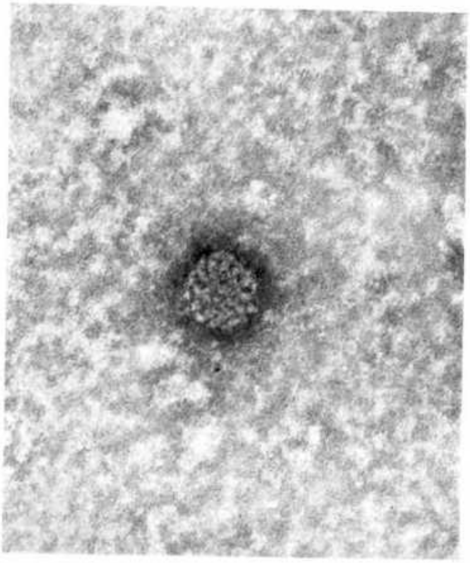

FIGURE 2. Papovavirus particle in homogenate of biopsy material (phosphotungstic acid negative stain, × 180,000).

of the cheeks, both lips, and on the sides of the tongue (Fig. 1). They were mostly discrete lesions, but some were confluent and they were less noticeable on stretching the mucosa. None were present on the floor of the mouth or the palate, and there were no warts at any other site on the body. These findings have remained unchanged during a follow-up period of over 2 years.

Investigations

Serological tests for syphilis were negative (VDRL, TPHA). Histology of an oral papule showed acanthosis and parakeratosis with prominent keratohyaline granules in some areas. There were some vacuolated cells present in the epidermis, a few dilated vessels in the papillary dermis, and the rete ridges were prolonged. These features are comparable to those described in other cases of focal epithelial hyperplasia. Papovavirus group particles were identified in tissue processed for electron microscopy and measurements of the diameter of two of these were found to be 59 nm (Fig. 2) and 61·6 nm. When processed for electron microscopy papovaviruses tend to flatten out, so that single particles often appear slightly larger than those closely packed together; this was taken into account in classifying them as belonging to the human papilloma virus sub-group (Colindale Virus Reference Laboratory).

DISCUSSION

There have been several different theories concerning the aetiology of focal epithelial hyperplasia. These have included local irritation or trauma in the mouth (for example from tooth-picks, or caustic spicy foods), nutritional deficiencies, and racial and genetic factors. Archard *et al.* (1965) in their original description noted a resemblance between the characteristic histological findings and those changes seen in epithelium infected by virus. Particles resembling the papovavirus group of viruses have been observed in the nuclei of superficial epithelial cells from oral lesions in a number of cases (Praetorius-Clausen & Willis, 1971; Hanks, Fischman & de Guzman, 1972; Van Wyk, Staz & Farman, 1977) and also in a chimpanzee who had the typical clinical and histological features of the condition (Hollander & van Noord, 1972). Two recent articles have reviewed the epidemiology and pathogenesis of focal epithelial hyperplasia, but no definite conclusion was made as to the role of the papovavirus in the aetiology (Starink & Woerdeman, 1977; van Wyk, 1977).

The papovaviruses are a family of small isohedral DNA viruses, the circular nature of the DNA being an important property of the group. The human papilloma (or wart) virus is a sub-group of large diameter (52–56 nm) and was the only known human papovavirus until 1965, although at this time four animal papovaviruses of a similar smaller size (41 nm) were known. Since 1965 new papovaviruses have been isolated from man, and an interest has developed as to their nature and significance in different diseases (Gardner, 1977).

Further advances have also been made in relation to the different morphological varieties of the human wart; in particular concerning its immunological aspects (Thivolet & Viac, 1978) and the oncogenic potential and heterogeneity of the human papilloma virus. In very recent studies, using biochemical DNA analysis and immunology methods, Orth *et al.* (1978) have identified two antigenically distinct human papilloma viruses, one isolated from common hand warts, the other from plantar warts. It has been further suggested that there may be four distinct types of human papilloma virus (Prunieras, 1978), each commonly associated with its own characteristic clinical presentation, namely: cutaneous warts and variants; ano-genital warts; florid oral and laryngeal papillomatosis; epidermodysplasia verruciformis.

In our case of focal epithelial hyperplasia the papovavirus particles measured 59 nm and 61·6 nm. In view of their large size they have been classified as belonging to the human papilloma virus sub-group. We therefore postulate that this virus may have a role in the aetiology of focal epithelial hyperplasia, and also may be another antigenically distinct type of human papilloma virus, producing its own unique clinical picture.

ACKNOWLEDGMENTS

We would like to thank Dr Anne M.Field (Colindale Virus Reference Laboratory) for providing information and the photograph of the papovavirus in Fig. 2; Dr K.Arul (now Consultant Pathologist, Farnborough General Hospital, Kent) and Dr R.W.Emmerson for reviewing the histology; and Mrs J.Walker for typing the manuscript.

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Cutis marmorata telangiectatica congenita in two sisters

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SUMMARY

Cutis marmorata telangiectatica congenita was observed in two adult sisters. The condition appeared at birth and did not change much with age. In one sister the condition was accompanied by hypertension, acrocyanosis and ulceration of the big toe.

On the basis of a comparison of the symptoms in patients and their relatives, the authors assume that Van Lohuizen syndrome is a dominantly inherited genetic disorder with low penetrance and great intrafamilial variability.

In 1922, Van Lohuizen described a child with skin lesions appearing as livedo reticularis, telangiectases and superficial ulceration. Histologically there were dilated capillaries, capillary and venous lakes and phlebectasia in the dermis and hypodermis. The child was followed up for 18 months. No visceral changes were noticed nor was the general condition of the child affected. Van Lohuizen decided that this was a benign skin blood vessel anomaly.

Since Van Lohuizen's communication about fifteen cases have been reported. Most of the patients were less than 1 year old at diagnosis and about ten were adults at the time of reporting.

The clinical manifestations, histopathology, possible pathogenesis and aetiological factors of Van Lohuizen syndrome have been extensively discussed in the papers of Way *et al.* (1974), Keipert & Nurse (1971), Bedell & Allison (1964), Lynch & Zelikson (1967), Moyer (1966), Fahrig (1968), Opitz, Hermann & Dieker (1969) and Laugier (1960).

The hereditary pattern however is still a controversial problem. In their review, Way *et al.* (1974) found that all cases of cutis marmorata telangiectatica congenita (CMTC) occurred as sporadic isolated instances in their families. In some members of their families there were other congenital anomalies, such as telengiectasis, keratosis pilaris, senile angiomas and extensive naevus.

We had the opportunity to observe the Van Lohuizen syndrome in two sisters. This observation and its genetic implication will be discussed in this paper.

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CASE REPORTS

Case 1

P.I.M., 32 years old. The skin lesions have been present since birth and she is positive that they have not changed since then. From the age of 13 years she noticed that her extremities become cold, livid and painful in cold weather. Her condition definitely improves in warm seasons. A small ulcer appeared recently on the right hallux, next to the nail, and has persisted in spite of treatment.

At the age of 16 years she developed hypertension of unknown origin. The blood pressure varies between 140/90 and 180/100 mmHg. In 1974 her condition was diagnosed as progressive scleroderma and a left brachial sympathectomy was performed. However there was no improvement either in the skin condition or in the vegetative symptoms (coldness, numbness and cyanosis of the extremities).

She has had three pregnancies resulting in stillbirths. An eclamptic toxaemia (a toxaemia of late pregnancy with hypertension, albuminuria and oedema) was suspected, but not proved.

Neurological and ophthalmological examinations did not reveal any abnormality. EEG was normal.

The family history is inconspicuous. The mother died at the age of 35 years (cause of death unknown); the father is still living and in good health. There were no other children except her and her sister. She is positive that there was no other member of the family (except her sister) with a similar condition. A sister of her mother apparently developed a great number of moles at the age of 40 (Fig. 1).

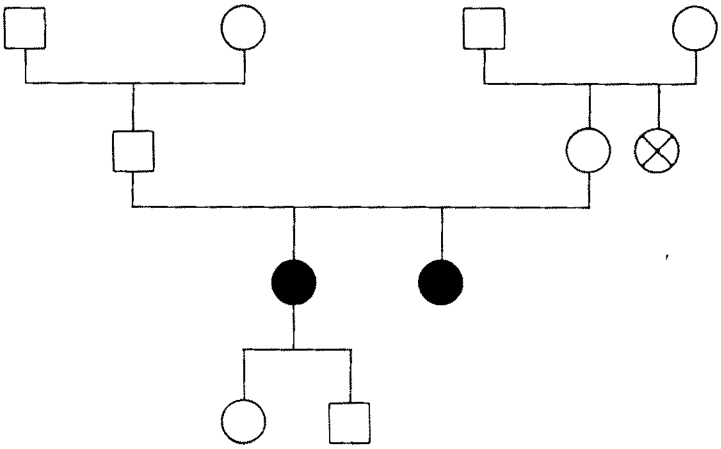

FIGURE 1. Family tree of the patients. (●) individuals with CMTC; (⊗) individual with other genetic anomalies; (□) males (healthy); (○) females (healthy).

Dermatological examination. The main clinical manifestation was livedo reticularis; the parts most involved being the thighs, gluteal region, arms and trunk (Fig. 2a and b). There was an intense facial erythema; the distal parts of the extremities were cold and cyanotic. On the right hallux, next to the base of the nail, there was an ulcer resembling a trophic ulcer.

Capillaroscopy of the nailfolds. The capillary network showed a regular pattern and the capillary loops had a palisade-like arrangement (which is normal). However, the venous segment of the loops was enlarged.

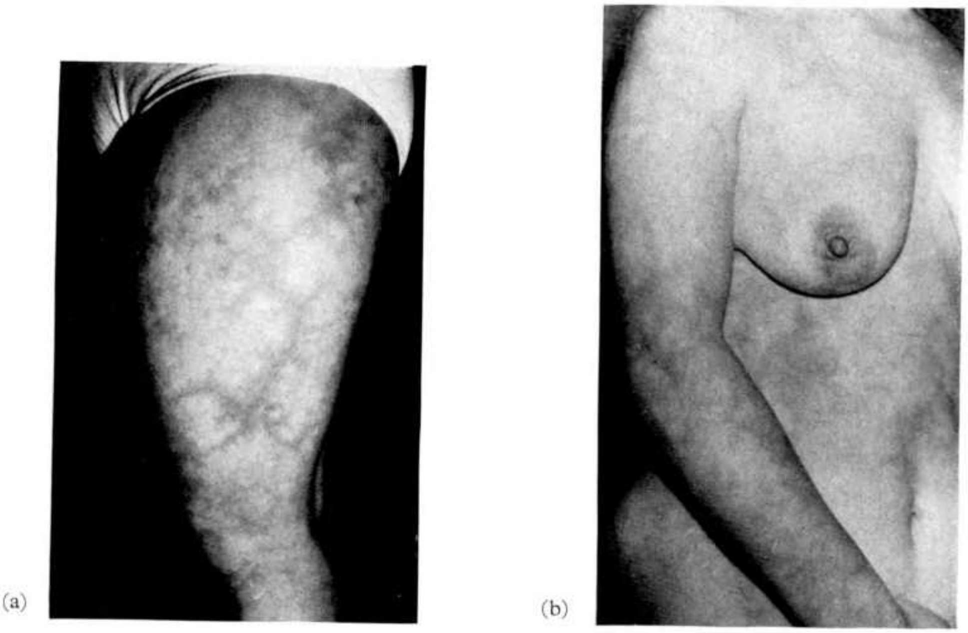

FIGURE 2. Case 1. Livedo reticularis-like telangiectases. (a) On the thigh; (b) on the arm and trunk.

Histopathology. The blood vessels in the upper and middle dermis were dilated, with swollen endothelial cells. There was no inflammatory infiltrate (Fig. 3).

The following investigations were normal: X-ray of chest and skull, WBC count, haematocrit, ESR, erythrocyte and platelet count, serum creatinine, serum electrolytes, creatine phosphokinase, urinalysis, cryoglobulins, cold agglutinations, transaminases. Tests for antinuclear antibodies, rheumatoid factor and LE-cell preparations were negative. No chromosomal anomalies were discovered.

Case 2

S.I.M., 29-year-old sister of Case 1.

The skin changes were present at birth and were considered initially as vascular naevi. They never bothered the patient and she never asked for medical advice.

At the age of 12 she had rheumatic fever with heart involvement, which later improved.

She is married and has two healthy children.

Dermatological examination. The skin lesions had the same appearance as in her sister, only they were milder and involved only the lower extremities. Livedo reticularis-like patches could be seen on the thighs and on the calves (Fig. 4), and stellate angiomata on the trunk.

All laboratory examinations were negative.

The histopathological findings are the same as in Case 1.

DISCUSSION

The clinical and pathological manifestations in both sisters are clearly in favour of the diagnosis

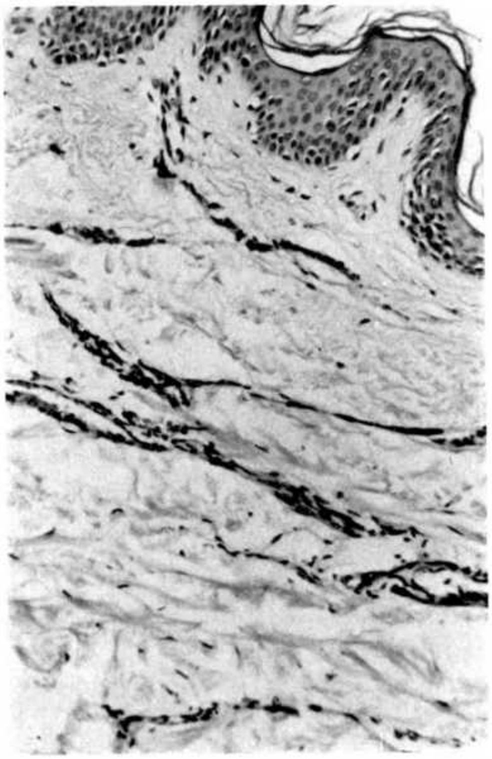

FIG. 3.

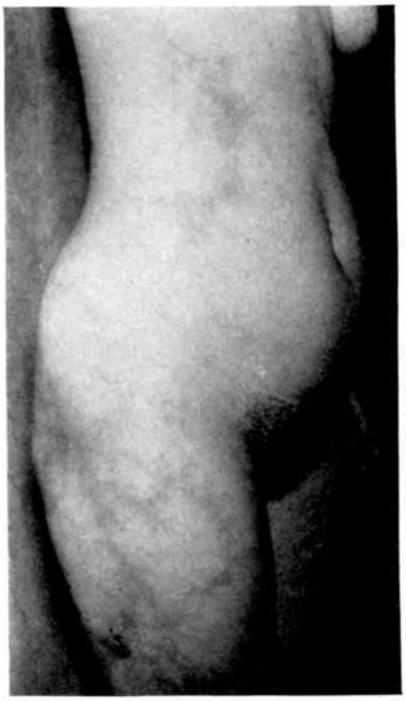

FIG. 4.

FIGURE 3. Case 1. Dilated blood vessels in the upper dermis with swollen endothelial cells.
FIGURE 4. Case 2. Livedo reticularis-like telangiectases on the thigh.

CMTC. In both of them the condition appeared at birth. At different times and by different physicians it had been considered as vascular naevus, livedo reticularis and cutis marmorata. In the elder sister (Case 1) hypertension, acrocyanosis and ulceration of the big toe were associated symptoms. Similar accompanying manifestations are not uncommon and have been reported by Petrozzi *et al.* (1970) and Pierini & Grinspan (1955).

As in most patients with CMTC the skin lesions did not improve very much with age and basically kept the same pattern as at birth.

Familial occurrence of Van Lohuizen syndrome has not been reported before to the best of our knowledge. Way *et al.* (1974) mentioned in their paper that a total of at least sixty-five siblings of reported patients have been unaffected. Two affected individuals had a total of eighteen children, all unaffected, and the family history has been negative in twenty-seven reports.

However, occasional minor and not always vascular manifestations in close relatives have been mentioned.

Waldecker (1937) reported that a 5-year-old sister of his patient had a palm-sized area of telangiectases between the scapulae, and varicose veins on the cheek, chin, trunk and knee; in addition she had keratosis pilaris on the extensor surfaces of the upper arms. Their mother apparently had senile angiomas.

The grandmother of Schwartzer's patient had an extensive naevus on her face. It is not mentioned, however, whether it was vascular or pigmented.

A number of malformations were noted in several members in the family of Case 1 of Way *et al.* (1974). The oldest sister had an irregular pigmented naevus over the left calf and an area of vitiligo over the left side of the abdomen; another sister had a cavernous haemangioma on the face; the mother showed a capillary haemangioma on her left buttock.

A paternal first cousin of Case 2 of Way *et al.* (1974) had hemihypertrophy.

There does not seem to be any significant relationship between these anomalies and the clinical manifestations of CMTC. However, if one takes into consideration the anomalies which accompany CMTC, and compares them with those observed in relatives of persons with CMTC, there do appear to be significant genetic implications (Table 1).

TABLE 1. Comparison between anomalies accompanying CMTC and anomalies observed in relatives of patients with CMTC

Symptoms observed in relatives of patients with CMTC	Number of patients with CMTC in which this symptom is present
Varicose veins	16
Keratosis pilaris	0
Senile angioma	1
Pigmented naevi	6
Vitiligo	0
Haemangiomata	18
Asymmetrical hypoplasia	21
Hypertrophy	2

As is evident from Table 1, symptoms such as varicose veins, haemangiomata, pigmented naevi, asymmetrical hypoplasia or hypertrophy (symptoms observed in relatives of proposita) are almost as much part of the Van Lohuizen syndrome as the telangiectatic changes themselves.

On the basis of this evidence, as well as on the basis of our cases, it may be suggested that CMTC is a dominantly inherited genetic disorder with low penetrance and great intrafamilial variability.

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Lichen striatus occurring as an annular eruption: an acquired 'locus minoris resistentiae'

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SUMMARY

A case of lichen striatus is reported in which the eruption occurred around the edge of a scar. It is suggested that the factors producing a 'locus minoris resistentiae' need not be congenital or linear.

Since the earliest description of lichen striatus, the pathogenesis of its linearity together with that of other linear dermatoses has been the subject of much discussion. Brocq (1898) put forward the hypothesis that a linear area of skin, a locus minoris resistentiae, could as a result of unknown congenital factors be prone to develop a dermatosis. There have been many attempts to relate the linear pattern to various anatomical structures and these have been reviewed in several papers (Senear & Caro, 1941; Staricco, 1959; MacDonald & Sims, 1969; Jackson, 1976).

Senear & Caro reviewed ten cases of lichen striatus, concluding that no single theory sufficed to explain all their cases. MacDonald & Sims reviewing seventy-five published case reports in which a good description or photograph of the distribution had been given, together with sixteen of their own cases, found no association between the lesions and any identifiable anatomical features of the skin; simple pharmacological tests of the superficial nerves and vasculature did not show any difference between normal and affected skin in eight of their patients with linear lesions (MacDonald & Sims, 1969). In this context the following case is of interest.

CASE REPORT

At the age of 16 months H.B. was severely scalded on the neck and upper part of his chest. The scald healed leaving a scar with keloid formation and a surrounding band of pigmentation. Two and a half years later he developed an eruption which arose suddenly and apparently unprovoked at the edge of the scar, forming a complete ring around it varying in width from 1–3 cm (Fig. 1). There was moderate itching. The lesion consisted of numerous erythematous lichenoid papules 2–3 mm across. There were no Wickham's striae. Three weeks after its onset the eruption faded. Histological examination of one of the papules showed a moderately dense mononuclear cell infiltrate around the capillaries in the superficial dermis extending into the dermal papillae and into the epidermis. The clinical picture most closely resembled lichen striatus, without of course its linear configuration.

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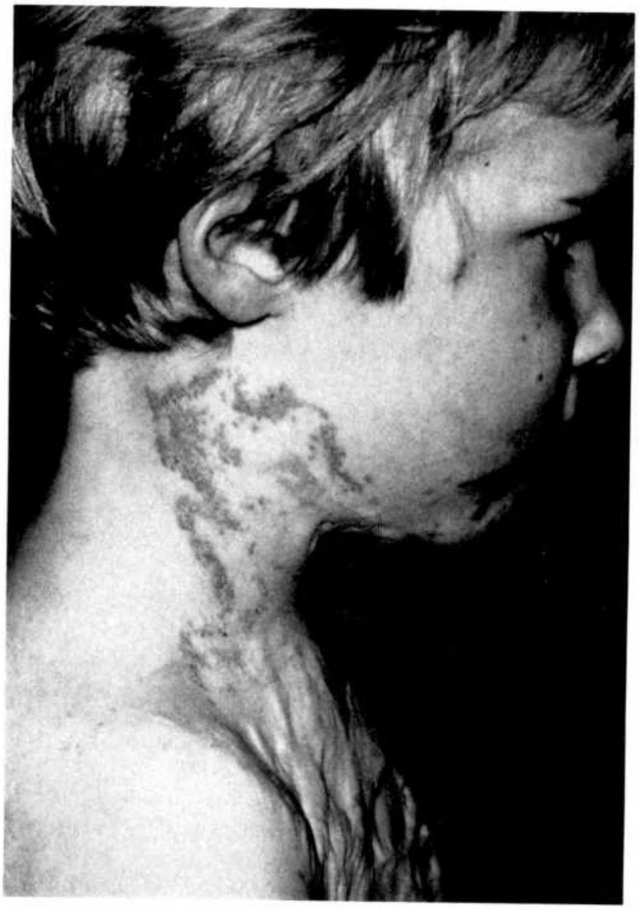

FIGURE 1. Lichenoid eruption encircling previous scar.

DISCUSSION

Much of the speculation surrounding the pathogenesis of lichen striatus and other linear dermatoses has invoked anatomical factors producing a congenital 'weakness' in the skin. The above case shows that the factors producing a 'locus minoris resistentiae' need not be congenital or linear but can occur as a result of other factors, in this case at the edges of an old scar.

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Primary extragenital disseminated cutaneous donovanosis

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SUMMARY

An extremely unusual case of non-venereal primary cutaneous donovanosis with distinctive evolution and morphology is described. Spread occurred by auto-inoculation and by direct continuity.

Donovanosis of the genitalia is a well-recognized entity in the tropics, including the Indian subcontinent (Rajam & Rangiah, 1954; Lal & Nicholas, 1971; Sehgal, 1978). While the large majority of these reports (Lal & Nicholas, 1971; Ramchander, Jaya Laxmi & Pankaja, 1967) are from southern states of India, stray cases have also been reported from other places. Three to six percent of cases are reported to have extragenital lesions as well (Rajam & Rangiah, 1954). Very occasionally, involvement only of organs other than the genitalia—extragenital donovanosis—has occurred. Oral donovanosis has been virtually the only type reported (Rajam & Rangiah, 1954; Lal, Arunthathi & Swahney, 1971; Ramchander *et al.*, 1967; Garg *et al.*, 1975).

We now report a very unusual case who had neither anogenital nor oral lesions.

CASE REPORT

B.L., a 25-year-old unmarried male from Rajasthan, reported with multiple shiny skin lesions of 10 years duration. The disease started with an egg sized firm swelling on the anterior aspect of the left side of the scalp. Repeated enquiries failed to reveal any history of prior trauma at the site. It was asymptomatic and became soft after about 20 days. There was a purulent blood-stained discharge after incision of the swelling. A U-shaped bandage was applied, but the discharge continued to trickle down the left side of the cheek and the neck for many days. Later similar eruptions were noticed along the bandage. He had some systemic as well as topical treatment the nature of which is unknown. He apparently recovered after 6 months treatment and remained well for about 2 years.

He then relapsed, but this time the lesions were at first confined to the legs. Their evolution was

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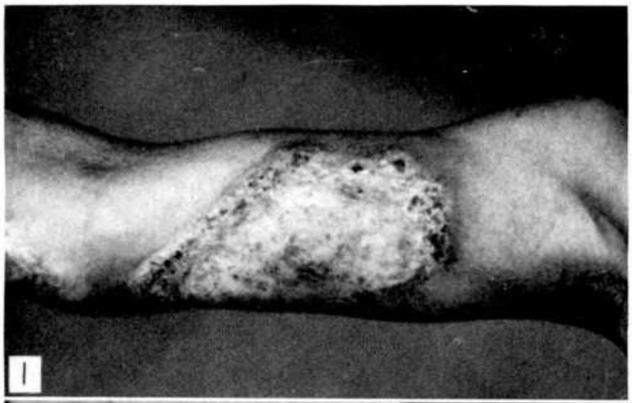
quite characteristic with a small asymptomatic swelling progressively extending with signs of central healing. Constitutional symptoms such as loss of appetite, fever, joint pains and weakness were conspicuous by their absence. Since then the disease had been progressing relentlessly, involving mainly the upper and lower extremities. Exacerbations occurred in summer. There was no history of sexual intercourse, or of lesions on the genitalia prior to or during the course of the disease in the patient, in his family or in the vicinity.

Examination

The skin was studded with multiple horse-shoe shaped, erythematous, indurated plaques varying in size from 4–30 cm. The lesions were active at the periphery and were marked by crusted papulopustules (Fig. 1). A bead of pus could be expressed on pressure. Shiny 'tissue paper' scars (Fig. 2) were the hallmark of healed lesions. They were present on the upper and lower extremities, and also on the face, where they were strikingly distributed over the cheeks and neck in a U-shaped fashion, corresponding to the original bandage. The lesions were asymmetrically disposed. The trunk and the mucous membranes were spared.



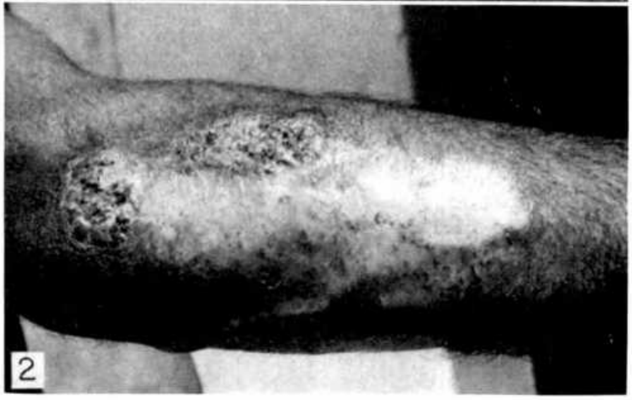


FIGURE 1. Crusted and papulopustular eruptions at the periphery of the plaques.

FIGURE 2. Healing plaque with shiny 'tissue paper' scar.

General physical examination including lymphatic, cardiovascular, respiratory and nervous systems did not reveal any abnormality.

Investigations

Routine blood examination revealed—Hb 10 g%; ESR (Wintrobe) 25 mm/h; leukocyte count 9,600/mm^3 (polymorphs 40%; lymphocytes 52%; eosinophils 2%; monocytes 2%); no abnormality was detected on macro- and microscopic examination of the urine; chest X-rays were normal; Mantoux test was negative; VDRL test was non-reactive; Lowenstein-Jensen culture for tubercle bacilli from the pus discharge was negative after 6 weeks.

Tissue smear. Reddish blue intra- and extracellular bodies were seen in Giemsa stained tissue smears prepared from the active periphery of the lesion.

Histopathological examination. Haematoxylin and eosin stained sections showed changes in both the dermis and epidermis. In upper dermis there was a dense cellular infiltrate consisting of large vacuolated mononuclear cells, plasma cells and scattered polymorphonuclear leukocytes. The mononuclear cells had multiple intracytoplasmic vacuoles, which were filled with faintly pink staining small round or elongated bodies. With Giemsa's stain, they were diagnosed as Donovan bodies because of their morphological characteristics and their presence in the vacuoles of mononuclear cells. Further confirmation came from silver stain (Warthin-Starry), with which these bodies showed a 'closed safety pin' appearance, a diagnostic morphological feature of Donovan bodies.

The epidermal changes were characterized by moderate to marked acanthosis with irregular elongation of rete ridges. Focal collections of cellular infiltrate were also seen in the dermal papillae and superficial epidermis.

Treatment and progress

Streptomycin 2 g daily was administered for 20 days. The response was excellent. There was a gradual regression of erythema and induration resulting in the formation of shiny atrophic scars. The tissue smears repeated after 10 and 20 days were negative for Donovan bodies.

DISCUSSION

The evolution and morphology were quite distinctive in our patient. There were certain other features which need emphasis; the spontaneous onset of the disease with no prior history of trauma or any sexual intercourse; the formation of lesions along the line of discharge; their multiplicity affecting primarily the upper and lower extremities, sparing the trunk, genitalia and mucous membranes; and a relentless progression for almost a decade. These features created a diagnostic dilemma. Several granulomatous conditions including lupus vulgaris, tertiary syphilis and dermal leishmaniasis were considered and ruled out by appropriate investigations. The demonstration of intracytoplasmic inclusions in the vacuoles of mononuclear cells in tissue smears, however, provided the diagnostic clue. The diagnosis was further confirmed by histopathology and identification of Donovan bodies by the silver impregnation method.

Our patient seems to be a clear example of non-venereal transmission of donovanosis and hence supplements the similar observations of other workers (Nair & Pandalai, 1934). Furthermore, the spread of the disease by auto-inoculation and by direct continuity is well illustrated. The involvement of the skin of the extremities and the multiplicity of the lesions were strikingly unusual, and such an example has not been reported so far (U.S. Public Health Service, 1964).

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British Journal of Dermatology (1979) **101**, 357.

Brief Communications

HLA and pemphigus

J. Zervas, A. Tosca, I. Apostolakis and A. Varelzidis *Department of Dermatology and Venereology, University of Athens A.Sygros Hospital, and First Department of Internal Medicine, University of Athens King Paul Hospital, Athens, Greece*

There is little information in the literature regarding HLA and pemphigus for patients of various racial and ethnic backgrounds. Katz *et al.* (1973) studying eighteen Caucasian patients of various ethnic backgrounds found an increased incidence of B13 ($P \sim 0·002$). Krain *et al.* (1973) found in forty-three pemphigus patients of different ethnic backgrounds an increase of A10 only in patients of Jewish origin. Hoshino, Inouye & Rajah (1976) demonstrated an association between HLA-A10 and pemphigus ($P < 0·001$) in nineteen Japanese patients.

In the present study, a panel of fifty-five HLA antisera have been used to determine twenty-four

TABLE 1. HLA specificities in pemphigus patients compared with controls

HLA-antigens*	Controls (Total = 280) %	Controls (Total = 280) No.	Pemphigus patients (Total = 20) %	Pemphigus patients (Total = 20) No.	χ^2	P	R.R.
A1	25·35	71	20	4	0·642	>0·1	0·735
A2	53·57	150	55	11	0·117	>0·1	1·05
A3	26·00	73	15	3	1·865	>0·1	0·50
A9	35·71	100	15	3	4·53	<0·05↓	0·31
A10	12·85	36	15	3	0·004	>1·6	1·196
A11	9·64	27	20	4	1·187	>0·1	2·34
A28	9·28	26	5	1	0·34	>0·1	3·25
AW19	7·96	18/226	—	—	—	—	—
A29	4·86	11/226	—	—	—	—	—
B5	32·50	91	25	5	0·88	>0·1	0·692
B7	13·21	37	40	8	9·14	<0·01↑	4·37
B8	12·50	35	30	6	3·47	<0·1	3·00
B12	18·21	51	25	5	0·20	>0·1	1·49
B13	10·35	29	0	0	3·63	<0·1	0
B14	7·85	22	15	3	0·48	>0·1	2·06
BW15	7·85	22	15	3	0·48	>0·1	2·06
BW16	4·71	10/212	—	—	—	—	—
BW17	8·57	24	0	0	3·20	<0·1	0
B18	29·64	83	—	—	—	—	—
BW22	4·76	11/231	35	7	20·95	<0·001↑	10·76
B27	6·42	18	15	3	0·99	>0·5	2·50
BW35	21·07	59	10	2	2·17	<0·1	0·41
BW40	7·52	17/226	15	3	0·67	>0·1	2·45
BW21	2·94	2/68	—	—	—	—	—

* Antigens AW19, A29, BW16, B18 and BW21 were not tested.

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HLA specificities of the A and B series in twenty patients with pemphigus and the results are compared to a control group of 280 healthy individuals (Table 1). The patients were all Greek Caucasians and were treated as in-patients in A. Sygros hospital. Some patients were on corticosteroid therapy. The HLA-typing was performed in the tissue-typing laboratory of the 1st Department of Internal Medicine, University of Athens, King Paul Hospital. The sera were kindly provided by the National Institutes for Health-Bethesda-Maryland and by Professor van Rood. For the identification of the HLA antigens on the surface of lymphocytes the two stage standard N.I.H. technique was performed. A significantly increased frequency of the antigen HLA-BW22 was found in the patients ($Pc^* < 0{\cdot}1$) and also a tendency towards increased frequency of HLA-B7 ($P < 0{\cdot}01$). The relative risks were 10·76 and 4·37 respectively. A tendency towards a decreased frequency of HLA-A9 ($P < 0{\cdot}05$) was noticed.

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* Adjusted to the number of antigens tested.

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Subcutaneous fat necrosis of the newborn and acute pancreatitis

T.A.J.DAWSON AND CLAIRE SLATTERY *Craigavon Area Hospital, Craigavon, Northern Ireland*

The patient was a female born in a general practitioner maternity unit 5 days after the expected date of confinement. Apart from meconium staining the birth was normal and uncomplicated. The baby weighed 3·4 kg at birth. Both parents and an elder brother aged 8 years were in good health. There was no history of diabetes mellitus in the family.

When 5 days old BCG vaccination was carried out on the infant's left arm That evening the skin at the vaccination site was erythematous and patches of erythema were also noticed on the other arm and on the tunk and hips. The infant became increasingly irritable and new skin lesions continued to develop. On the seventh day she was transferred to the special-care baby unit, Craigavon Area Hospital. At that time the infant's cry was abnormal as though she were in pain. Multiple dusky red lesions were present on the arms, back, hips and thighs. The surfaces of the lesions were level with the surrounding skin but on palpation there was firm underlying induration. The lesions varied from 0·5 to 2 cm in diameter. The largest lesion coincided with the BCG vaccination site. The clinical diagnosis was subcutaneous fat necrosis of the newborn. Nothing definitely abnormal was found on systemic clinical examination though supporting the patient so that she was leaning forwards appeared to relieve her pain.

No special treatment was given. There was little change in the patient's condition for about 10 days. At the end of that time a distinct improvement occurred. A pyrexia which had reached 38°C settled, the cry returned to normal and skin lesions began to resolve.

During the acute phase after admission to the special-care baby-unit investigations gave the following results: Hb 17·1 g/dl; WBC $15{\cdot}2\times 10^9$/l; neutrophils, 38%, lymphocytes, 54%, monocytes 4%, eosinophils, 4%; VDRL and TpHA negative; blood sugar 4·5 mmol/l; serum amylase greater than 500 Street-Close units/100 ml (normal range 15–35 Street-Close units/100 ml).

The infant was discharged aged 28 days by which time her general condition was good, the skin lesions were still present but much less conspicuous and the serum amylase had fallen to 14 Street-Close units/100 ml.

The patient continued under review for a further 9 months at the out-patient clinic. Two months after discharge all the skin lesions had disappeared except the one at the BCG site. This broke down and discharged pus from which tubercle bacilli were cultured. Eventually the discharge stopped leaving slight residual induration. No fresh lesions of subcutaneous fat necrosis of the newborn appeared, the patient's health remained good and development progressed satisfactorily.

It is possible that subcutaneous fat necrosis of the newborn and pancreatitis were associated by chance in this patient. On the other hand the synchronous clinical improvement, both in the skin lesions and general condition, with the fall in the serum amylase suggests that they were related.

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Independent lesions of fixed eruption due to two unrelated drugs in the same patient

JAGJIT S.PASRICHA AND SHAILJA R.SHUKLA *Department of Dermato-venereology, All India Institute of Medical Sciences, New Delhi, India*

Cross-sensitivities between various drugs causing fixed eruptions are well known (Welsh, 1952, 1955, 1961; Dougherty, 1952; Browne, 1964; Fitzpatrick, 1965; Tarnowski, 1970; Nayyar & Pasricha, 1972; Pandhi & Bedi, 1975; Pasricha, 1978) and poly-sensitivities have also been recorded (Welsh, 1961; Pasricha, 1978). But occurrence of two groups of lesions in the same patient, one group reacting to one drug and the other group to another unrelated drug, is rare. There is only one report of a patient reacting to arsphenamine and phenobarbitone (Chargin, 1938). We are reporting a case reacting to Analgin (metamizole) and tetracycline.

CASE REPORT

A 32-year-old male developed repeated attacks of fixed eruption after taking metamizole during the first episode, an unknown tablet during the second episode and tetracycline during the third. During provocation tests, he showed reactivation of the lesions following oral administration of one tablet (500 mg) of metamizole. Subsequent provocation tests with three more drugs, acetyl salicylic acid, vitamin B complex and sulphadiazine, did not reactivate the lesions, but one capsule (250 mg) of tetracycline led to the appearance of three lesions of fixed eruption on the chest. None of the other previous lesions showed any sign of activity. Approximately 1 week later, challenge with 10 mg metamizole led to reactivation of all the old lesions except those caused by tetracycline and 2 weeks later, 75 mg tetracycline resulted in reactivation of only those lesions caused by tetracycline.

DISCUSSION

Our case is distinct from instances of cross-sensitivity between two drugs which have a common antigenic determinant and all the lesions are reactivated following administration of either of the drugs. Our case is to be distinguished also from cases of poly-sensitivity where all the lesions respond to two or more unrelated drugs. The case with independent lesions caused by two unrelated drugs does not require any special explanation, but confirms that the factor(s) which determine localization and recurrence of lesions on the skin are very specific.

In every such case, it is necessary to provoke at least twice with each drug to ensure that each drug activates only its own group of lesions.

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British Journal of Dermatology (1979) 101, 363.

Correspondence

Unacceptable side-effects of oral zinc sulphate in the treatment of acne vulgaris

Sir, There has been recent interest in the use of oral zinc in the treatment of acne vulgaris (Michaëlsson, Vahlquist & Juhlin, 1977; Michaëlsson, Juhlin & Ljunghall, 1977; Hillstrom *et al.*, 1977) and this was reviewed in a leading article in the *British Medical Journal* (1977).

During the past 2 years, in an open-ended study, I have treated forty-two patients who have had acne of moderate severity, with zinc sulphate 220 mg three times daily, the tablets taken after food. This preparation, unlike that used by the Swedish workers, was not citrated. Thirteen of the patients had to stop therapy because of undesirable side-effects. In ten cases the subjects developed severe nausea and vomiting and two developed severe diarrhoea, three times daily, but not associated with pus, blood or slime. Twelve patients had to stop therapy within 1 week of instituting treatment. On request they re-took the zinc sulphate and within a few days developed the same side-effects. The thirteenth patient, a 21-year-old male student who had been taking zinc sulphate for 4 months, developed perforation of a previous asymptomatic gastric ulcer, requiring surgical treatment. This is the second report from the United Kingdom of gastro-intestinal ulceration as a result of zinc therapy (Moore, 1978). Of the twenty-nine patients who were able to tolerate the zinc sulphate for 4 months, only in three patients was there any detectable clinical improvement.

This open-ended study fails to confirm the value of oral zinc sulphate in the treatment of moderate acne and demonstrates that zinc sulphate in the absence of its citrate complex is, in the acne age-group, a poorly tolerated drug, producing marked gastro-intestinal problems which in one patient was life threatening. Thus, our recommendation is that zinc sulphate should not be used orally in the treatment of acne.

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British Journal of Dermatology (1979) **101**, 364.

Book Reviews

Sexually Transmitted Diseases, 3rd edn. By C.B.S.Schofield (1979) Edinburgh: Churchill Livingstone Medical Text. Pp. 249. Price £3.25.

I was brought up in the paperback age and can still remember the pleasure that this new freedom—the ability to buy books for oneself—gave me. Sadly, many years later, I still find that the paperback is the only book that I can afford—at least in the quantities that I like to buy. Thus, respectable as the paperback or 'soft cover' has now become, I had never been able to take it very seriously where medical books were concerned—these volumes after all have to stand up to a lot of hard work, constantly being referred to, often hurriedly and perhaps without the care that a formal reading usually ensures. The first edition of Dr Schofield's book was the first medical text to convince me of the error of my ways: it has been much used and has stood up to the test well. The appearance of a third edition in 5 years attests to its qualities other than durability. I like the book because it is readable and, furthermore, deals comprehensively if not exhaustively with its subject, the sexually transmitted diseases.

A good account is given of the epidemiology and control of these infections, a subject all too often omitted or dealt with in an off hand fashion in text books of this size. The 'old fashioned' venereal diseases, syphilis and gonorrhoea, are carefully dealt with and reference is made to the most recent work. (A most useful feature, especially for the postgraduate student, is the inclusion at the end of each chapter of a carefully chosen list of references to important papers, articles and monographs.) There is an especially clear accurate account of modern serological tests for syphilis and their clinical use. Whilst there is not much room for critical surveys and dogma seems to be the order of the day in all text books, especially small ones, perhaps some reference should have been made to the fact that there is a school of thought which does not think it necessary to examine the cerebro-spinal fluid after completing treatment for early infectious syphilis, nor to examine three specimens of the prostatic secretions in a patient apparently successfully treated for gonorrhoea.

Elsewhere non-specific infections, Reiter's Disease, viral sexually transmitted diseases and genital candidosis are efficiently dealt with, though the statement that herpetic lesions are rarely seen on the cervix in genital herpes is hard to accept. Chapters on tropical diseases affecting the genitalia, the tropical treponematosis and the common dermatoses of the anogenital region, give some idea of the wide coverage achieved.

In general terms, this is a very good book which can be wholeheartedly recommended to undergraduate and postgraduate students as well as those working in allied specialties. What is specially nice about it is that a confident recommendation to buy it, as well as to read it, can be made at £3.25 which is indeed a bargain.

J.K.Oates

Behçet's Disease. Edited by Nihat Dilsen, Meral Konice and Candan Övül (1979) Amsterdam: Excerpta Medica. Pp. 308, B/W illustrated. Price $61 or 125 Dfl.

This handsome volume publishes fifty papers which were presented at the International Symposium on Behçet's disease in September 1977 at Istanbul. It is well edited and produced, and provides an easily read summary of current knowledge and thereby a 'Who's Who' of scientists working on the problem. Quite clearly the subject is wide open for more research and this book will help point the ways. I conjecture that Behçet's original guess (1937) of an infective agent as a triggering factor, and he suggested a virus, is likely to prove correct. If the association recently being mooted of herpes simplex infection with uveitis is substantiated, then his hunch will have been gloriously upheld. In which case I'll concede defeat in my small campaign over the last couple of decades to rename the disease after our own Whitwell (1934)!

P.W.M.Copeman

Clinical Skin Microbiology. By R.ALY and H.I.MAIBACH (1978) Illinois: C.C.Thomas. Pp. 133, B/W illus, 6 col. plates. Price $14.50.

The intention behind this book is to provide a do-it-yourself manual for cutaneous microbiologists. To the absolute tyro it will prove valuable in presenting the common laboratory manipulations between two covers. Some of the colour plates are helpful (that of the beta haemolytic streptococcus is excellent); others are less helpful even to the more experienced eye. There are two main deficiencies; the first, as the authors acknowledge, is that mycology has been left out (but virology included) on the grounds that other suitable books exist. This would seem to lessen the book's value as a single handbook. The second is that the authors have not got to grips with modern taxonomy. The section on Micrococcaceae entirely ignores recent speciation of *Staphylococcus* which, even if it is a complex matter, is proving of epidemiological value; that on the Gram negative bacilli still separates 'Mima' and 'Herellea' both now regarded as a single genus Acinetobacter (Aly & Maibach's own Table 7.1 shows why this should be so!). From two such experienced workers, this book disappoints.

W.C.NOBLE

Candida and Candidosis. By F.C.ODDS (1979) Leicester: Leicester University Press. Pp. 382. Price $16.00.

This book is a worthy successor to that of Winner and Hurley. It covers the biology and classification of *Candida* species, their ecology and epidemiology; each area of the body is discussed separately. Antifungal drugs, in which the author has a particular interest, are well described including the more recent variants of established antifungal agents. There are sufficient high quality illustrations, both clinical and microbiological, to illuminate the text. Much data has been collected into useful tables, for example in that on disorders associated with muco-cutaneous candidiasis it is quickly apparent that some 101 patients have been the subject of sixty-two publications. The references are numbered to facilitate reading the text and appear at the end of the book (all 2265 of them). If there is a criticism it must be that the book has been more than a year in press so that it is light even on 1977 references; the author deserved better treatment. Nevertheless this is a most useful and interesting book which will remain the standard work until the second edition is published.

W.C.NOBLE

Office Techniques for Diagnosing Skin Disease. By WILLIAM H.EAGLSTEIN and DAVID M.PARISER (1979) Chicago: Year Book Medical Publishers. Pp. 194, B/W illus, col. plates. Price £19.50.

In the foreword, Professor Harvey Blank wonders whether the reason why so many medical students often overlook the skin is perhaps the fact that they are taught that the heart and breath sounds are best heard with the eyes closed! As a clinical laboratory manual of the skin, this book bridges the gap between the patient with a skin lesion and a textbook account of the disease.

Diagnostic techniques are simply described and illustrated. Not only are the purpose, diagnostic value and clinical indications of a Wood's light examination succinctly given, but we are also told what kinds of Wood's lamp exist and where they can be bought. Patch testing, cytodiagnostic procedures, biopsies, darkfield examination, immunofluorescent testing, ectoparasite demonstration and diagnostic manipulations are clearly described and supported with superb colour and monochrome pictures. Collection of scales for potassium hydroxide examination from the skin is demonstrated precariously using a scalpel with a detachable blade. The banana shaped knife used in many skin departments in this country for this purpose is both safer and more efficient and I recommend it for inclusion in the next edition. This timely and attractive booklet is a must for all who deal with the skin, including dermatologists, physicians, general practitioners, casualty officers, technicians and students.

I.SARKANY

Progress in Allergy, Vol. 26. Edited by KIMISHIGE ISHIZAKA, PAUL KALLOS, BYRON H. WAKSMAN and ALAIN L. DE WECK (1979) Basle: S. Karger. Pp. 238, 17 figs, 40 tables. Price SFr.99; U.S. $59.50.

This is a very up-to-date account of the scientific basis of allergy. It contains chapters in relation to cross-reactions between mammalian tissues and streptococci, a topic which has interested dermatologists for many years. It has also interesting chapters on the relation of immunological phenomena to tumour behaviour and indicates the scientific background of this work as shown by experimentation in nude mice.

I do not consider that it will be of great value to many dermatologists but I am equally sure that if a dermatologist has an interest in subjects impinging upon allergy, he will find it a most useful and reliable reference book and introduction to the latest technology.

C.N.D. CRUICKSHANK

British Journal of Dermatology (1979) **101**, 367.

News and Notices

Welsh National School of Medicine

The third course on the Investigative Techniques in Dermatology will be held at The Welsh National School of Medicine, Cardiff, 10–12 October 1979.

The course is designed to give registrars and senior registrars insight into the basic research techniques applicable to dermatology, and into some of the newer techniques for investigating patients with skin disorders.

Anyone who is interested should write for further details to: Mrs J. Hayes, Secretary to Dr R. Marks, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN.

Investigative Group Meeting, British Association of Dermatologists, 18–19 January 1980, Glasgow

This meeting will take place on the afternoon of Friday, 18 and the whole of Saturday, 19 January 1980, at the Department of Dermatology, University of Glasgow.

Please forward abstracts to: Professor R.M. MacKie, Department of Dermatology, Anderson College Building, 56 Dumbarton Road, Glasgow, G11 6NU, not later than 31 October 1979. (Abstracts not received by this date will not be considered.)

Seven copies of the abstract should be typed double-space on 8·5 × 11 inch or 21 × 29·5 cm paper. The abstract should consist of *not more than* 250 *words* and should contain a statement of the purpose of the study, methods used, and a summary of the results presented in sufficient detail to support the conclusions. Supplementary information, such as electron micrographs and tables, can be submitted at the discretion of the author for the use of the selection committee. Please underline name of author who will present the paper.

Accommodation, at a reasonable price, will be arranged.

There will be a subsidized meal on the evening of Friday, 18 January 1980.

Full details of the meeting will be circularized to members of the B.A.D. and the Investigative Group later in the autumn. Others interested should contact Professor R.M. MacKie. The meeting is open to clinical and non-clinical colleagues.

Dermatology Course for State Enrolled Nurses

A post graduate course organized by the Joint Board of Clinical Nursing Studies will be held at Beaumont Hospital, Lancaster, for 6 months starting 24 September 1979.

Further information may be obtained from the Skin Department.

The Royal College of Physicians of Edinburgh

The following dermatologists have recently been elected to the Fellowship of the Royal College of Physicians of Edinburgh: Dr P.B. Haribhakti and Dr J.A. Savin.

Journal of the American Academy of Dermatology

A new journal, the *Journal of the American Academy of Dermatology* will be appearing monthly from July 1979.

It is the official publication of the American Academy of Dermatology. The editor is Dr J. Graham Smith, Jr, Department of Dermatology, Medical College of Georgia, Augusta, Georgia.

The publishers are the C.V. Mosby Company, Journal Circulation Department, 11830 Westline Industrial Drive, St Louis, Missouri 63141, U.S.A.

University of Cambridge

The Annual Introductory Course on the Biology of the Skin will be held in Fitzwilliam College, Cambridge, on 11–14 December 1979.

Details may be obtained from The Postgraduate Medical Centre, Level 6, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ.

The Netherlands Association of Dermatology and Venereology

The next meeting of the Netherlands Association of Dermatology and Venereology will be held on 27 October 1979 at Erasmus University, Dijkzigt Hospital, Rotterdam.

Further information may be obtained from Dr S.M.C.Molhuysen-Van Der Walle, Guido Gezellestraat 6, Amsterdam-Z.11, The Netherlands.

Corrigenda

In the letter 'Effect of progesterone on sebum excretion rate' by N.B.Simpson, D.Dick and M.B. Hodgins (*British Journal of Dermatology* (1979) **100**, 735) the following important sentence was omitted and should appear after the second paragraph: 'The results showed that at the end of 2 months there was no improvement in either group'.

Protease inhibition profiles in urticaria and angio-oedema by N.Eftekhari, A.Milford Ward, R.Allen and M.W.Greaves (*British Journal of Dermatology* (1979) **101**, Suppl. 17, 20).

The text in the above abstract should have read as follows: 'Values for cold urticaria patients showed a mean reduction in $\alpha 1$ anti chymotrypsin level ($0{\cdot}47 \pm 0{\cdot}11$ g/l) compared with comparable values of $0{\cdot}56 \pm 0{\cdot}15$ in the control group ($P < 0{\cdot}05$)'.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Presentation
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Indications
For the topical treatment of subacute and chronic psoriasis including psoriasis of the scalp.

Direction
Apply sparingly to the psoriatic lesions preferably at night and rub in gently and carefully until absorbed. Bathe before the next application. For scalp lesions, shampoo before use. Continue treatment until the skin is smooth and clear.

Contraindications
Acute psoriasis; dithranol sensitivity.

Precautions
Keep away from the eyes. Apply to psoriatic areas only. Use cautiously after recent application of potent steroids. Wash the hands after use.

Prices
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Product Licence No.
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Telephone 01-580 0875

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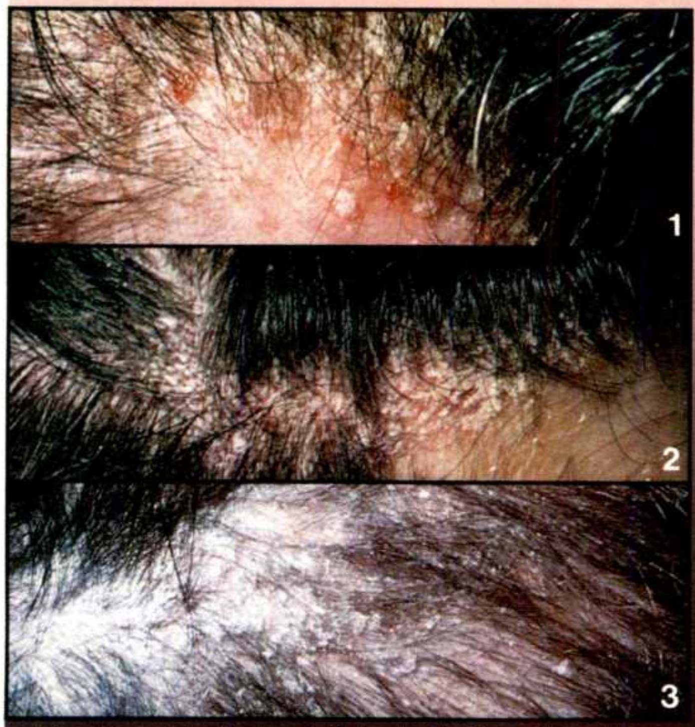

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Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
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	1 litre	£3.90
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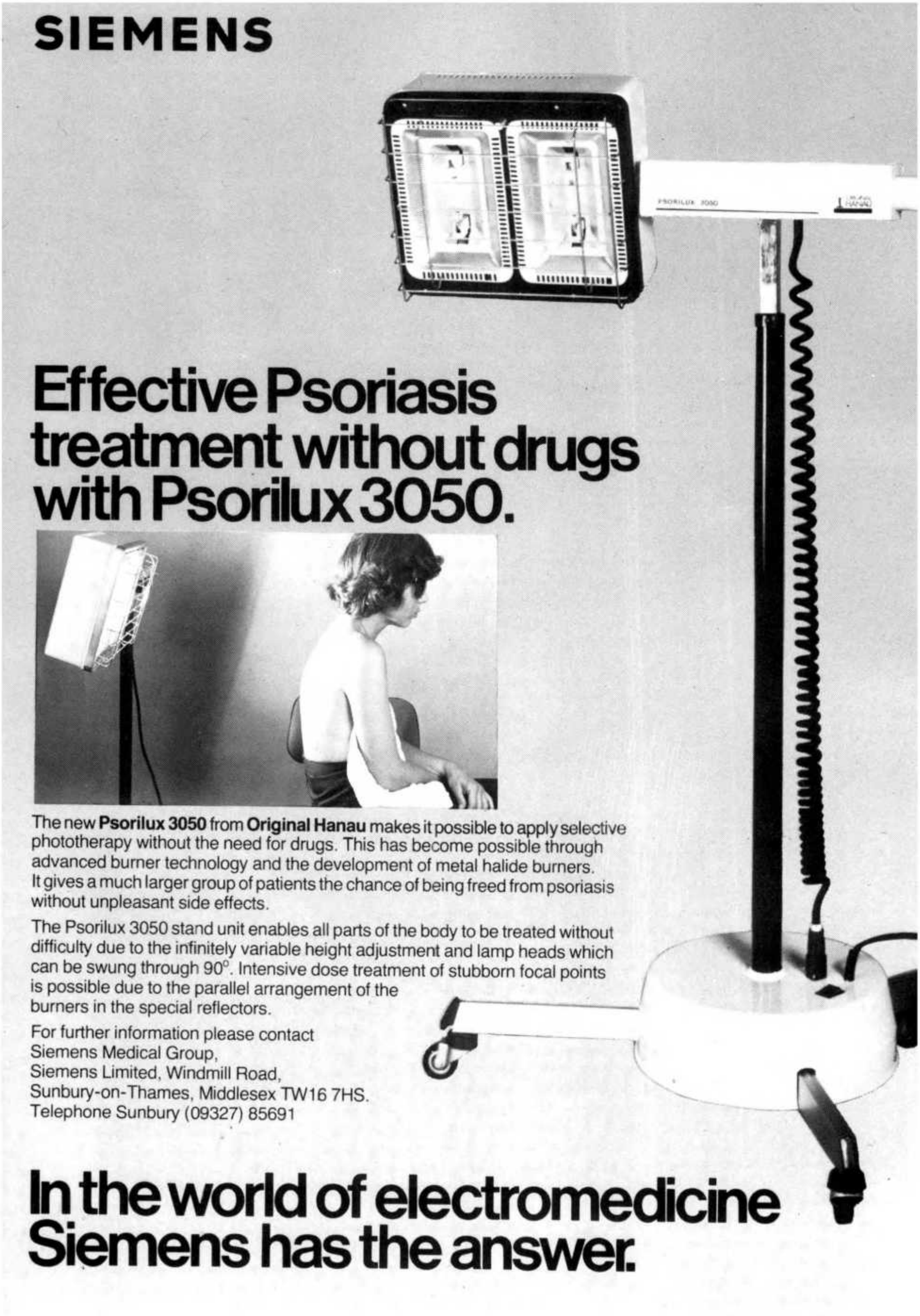

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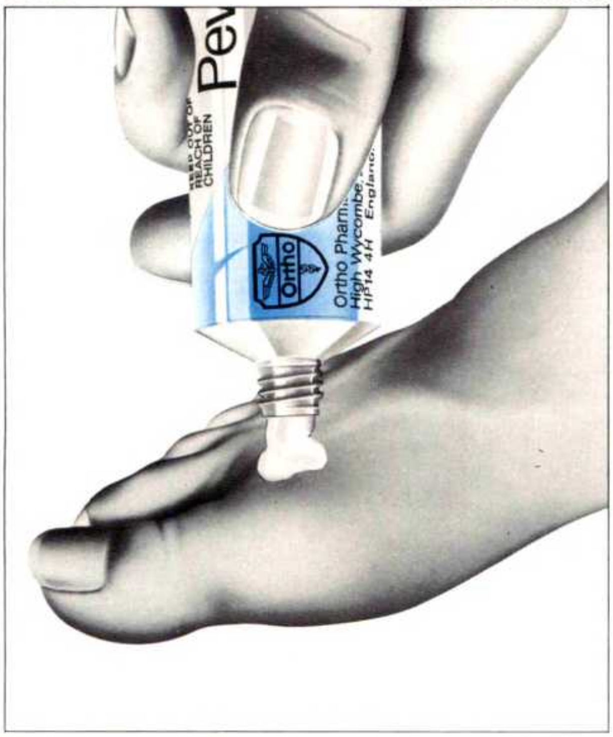

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1. Thienpont, D. *et al* (1975) Arzneim Forsch. **25**, 224-230. 2. Raab, W. (1977) Curr Ther Res. **22**, 65-82. 3. Dorn, M. *et al* (1975) Munch. Med. Wochenschr. **117**, 687-692.

Every Inch a Fighter

Full information from:
ORTHO PHARMACEUTICAL LIMITED,
Saunderton, High Wycombe, Buckinghamshire HP14 4HJ

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Prescribing Information:

Presentation:
Cream: A white cream containing 1% econazole nitrate.
Lotion: A white water-miscible lotion containing 1% econazole nitrate.

Uses:
Econazole nitrate is a broad-spectrum antifungal agent.
Cream: All fungal skin infections due to dermatophytes (e.g., Trichophyton species), yeasts (e.g., Candida species), moulds and other fungi. These include ringworm (tinea) infections, athletes' foot, paronychia, pityriasis versicolor, erythrasma and vulvitis, balanitis and napkin rash due to Candida.
Lotion: As PEVARYL* Cream, but particularly suitable for hairy and moist areas and for fungal otitis externa.

Dosage and Administration:
Cream: Apply to the affected area 2-3 times daily and rub in gently.
In nail infections, apply once daily and cover with an occlusive dressing.
Lotion: As for PEVARYL* Cream.
In order to prevent relapse, treatment should be continued for 2 weeks after clinical cure.

Contra-indications, Warnings, etc:
Contra-indications: None known.
Side-effects: Rarely, transient local mild irritation may occur immediately after application.
Overdosage: Not applicable.
Precautions: Hypersensitivity has never been recorded but if it should occur, administration of the product should be discontinued.

Pharmaceutical Precautions:
Storage: None.

Legal Category:
Cream: Prescription only (POM).
Lotion: Prescription only (POM).

Package Quantities:
Cream: Each tube contains 30g.
Lotion: Each bottle contains 30ml.

Further Information:
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Cream: UK: PL 76/59.
Republic of Ireland: PA59/9/3.
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P. L. 0102/0005. Further information and data sheets from Bengue & Co Ltd. St. Ives House, St. Ives Road, Maidenhead, Berks SL6 1RD.

[1] Hanna, N. F., Clay, J. C., Harris, J. R. W., Br. J. Vener. Dis. 1978, **54**, 354.
[2] Syntex Pharmaceuticals Limited. Data on file *DERBAC is a trademark.

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Precautions

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Appropriate chemotherapy should be used whenever infection of the skin is present. Any spread of infection requires withdrawal of topical corticosteroid therapy.

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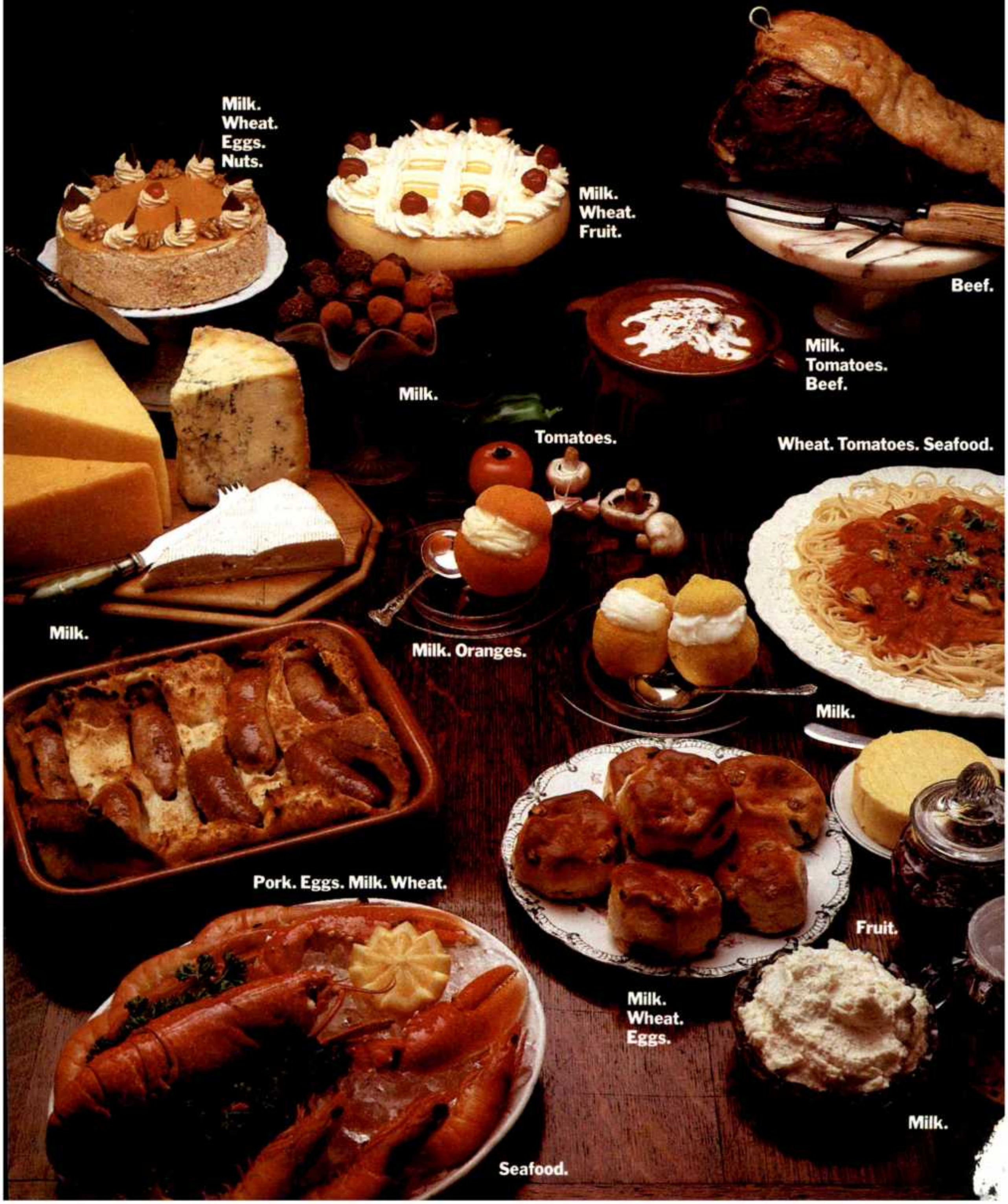

And so, at last, does an effective drug treatment.

The whole business of food allergies admittedly, a difficult, often unclear and metimes contentious one.

DIAGNOSIS:THE BASIC PROBLEM.

The symptoms of food allergies may cur in the gastro-intestinal tract, mimic seases in other systems.

Like, for example, chronic diarrhoea ıd other gastro-intestinal symptoms), ticaria and eczema.

The exact mechanism is uncertain.

But it appears that initially the allergen uses a reaction in the wall of the gut.

This, in turn, leads to gastro-intestinal mptoms or, indirectly, symptoms in other rget' organs.

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The obvious way to treat food allergies of course, to eliminate offending foods.

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FISONS Leaders in Allergy Research. Fisons Limited, Pharmaceutical Division, Derby Road, Loughborough, Leicestershire LE11 0BB. **Dosage and ninistration:** (a) Chronic inflammatory bowel disease as an adjuvant in the treatment of ulcerative colitis, proctitis and proctocolitis – two capsules four times y in adults and one capsule four times daily in children from 2-14 years, before meals. (b) Food allergy – as above for the initial dose. If satisfactory control is not ved within three weeks, the dosage may be doubled but not exceed 40mg/kg/day. Dosage may be reduced to the minimum required to maintain the patient free toms. Protection may be afforded by a single dose taken 15 minutes before a meal in which there may be an unavoidable allergenic food. The capsules may wed whole or the powder contents may be dissolved in hot water and diluted with cold water to drink. **Contra-indications:** There are no specific contra-s. **Warnings:** The safety in pregnancy and the treatment of children under two years has not yet been established. **Side effects:** Nausea, skin rashes and have been reported in a few cases. **Over-dosage:** No action other than medical observation should be necessary. **Basic NHS cost:** £17.14 per 100 capsules.

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073.

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1. Munro, D.D., Wilson L.C., *British Medical Journal* (1975) **3,** 626
2. Munro, D.D., *Journal of Dermatology* (1976) **94** (Suppl.) 12 67

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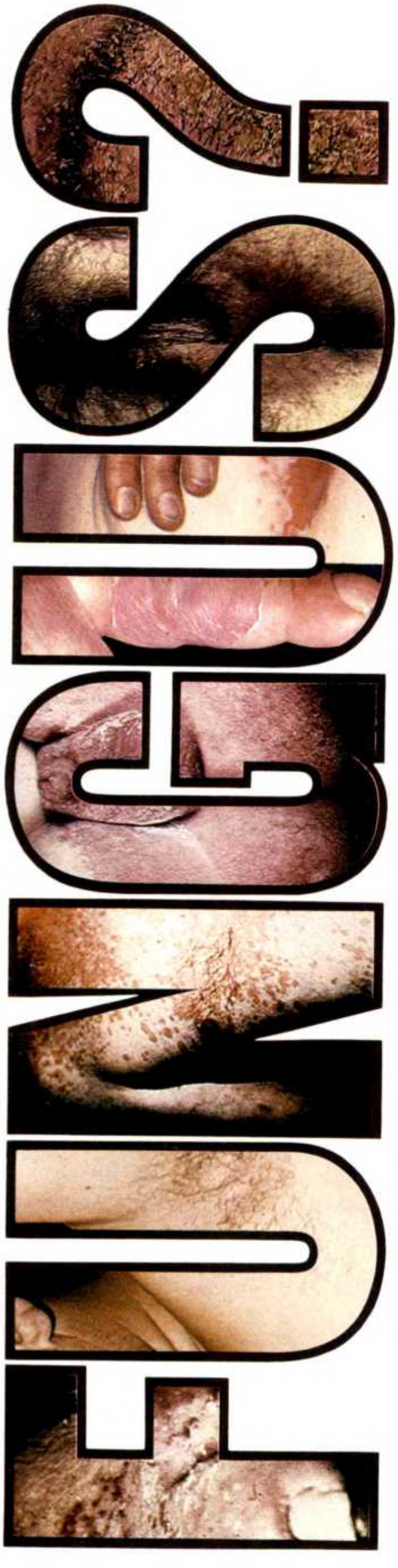

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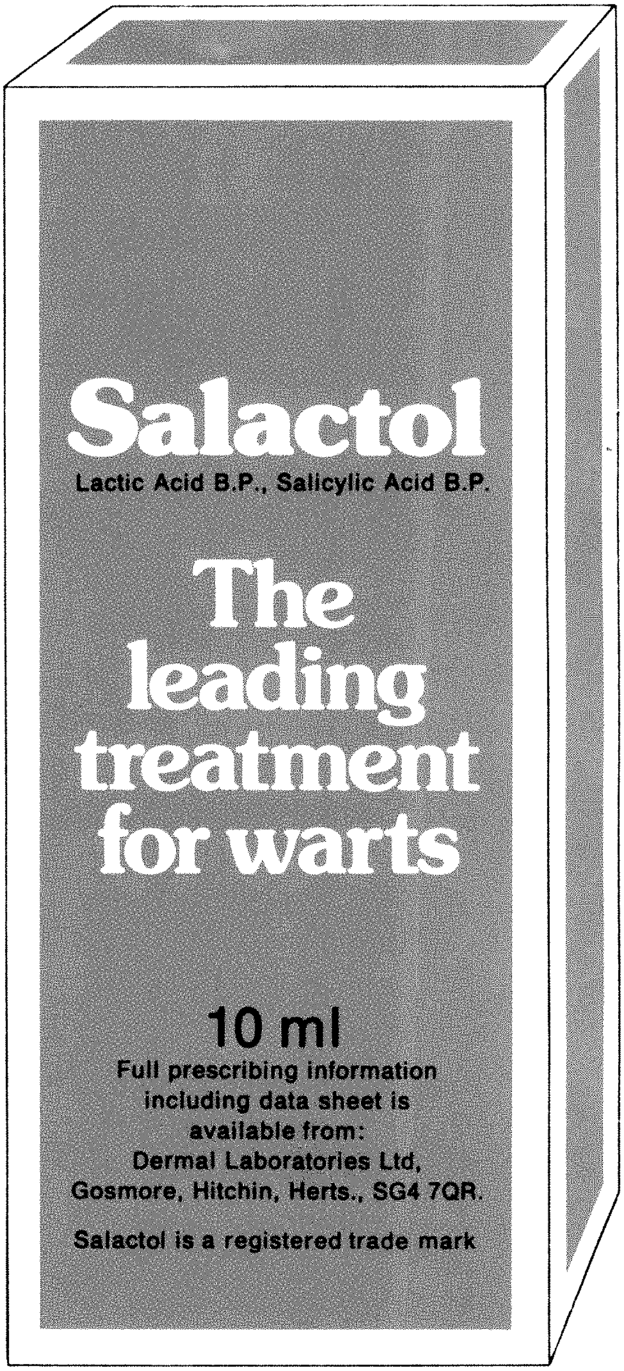

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Clinical and Laboratory Investigations

5-Methoxypsoralen (Bergapten) in photochemotherapy of psoriasis

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SUMMARY

5-Methoxypsoralen (5-MOP, Bergapten) was evaluated as a potential photosensitizing drug in oral photochemotherapy of psoriasis. Treatment results indicate that (1) 5-MOP is as effective as, and in high doses more effective than, 8-methoxypsoralen in clearing psoriatic lesions; (2) therapeutic doses of 5-MOP do not lead to erythema; the acute side-effects of 8-MOP PUVA therapy—erythema, blistering, pruritus—are thus avoided; (3) even high doses of 5-MOP are not followed by nausea. 5-MOP PUVA therapy thus represents a real alternative to 8-MOP PUVA, its advantages over 8-MOP PUVA being greater safety and patient acceptance.

Photochemotherapy (PUVA) which is based on the combined use of orally administered 8-methoxypsoralen and subsequent long wave ultraviolet (UV-A) irradiation (Parrish *et al.*, 1974) has been shown to be a highly efficient treatment for severe psoriasis (Wolff *et al.*, 1975, 1976). One of several possible approaches to improve PUVA even further is the replacement of 8-MOP by another, possibly better, psoralen derivative in the PUVA regimen. To this end, we tested a naturally occurring psoralen analogue, 5-methoxypsoralen (Bergapten), for its photosensitization capacities, its efficacy in PUVA treatment of psoriasis, and its side-effects, and compared this regimen to standard 8-MOP PUVA treatment.

MATERIAL AND METHODS

Phototoxicity tests

The tests were performed in psoriatic and non-psoriatic human volunteers, who received oral 5-MOP†

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† 5-MOP (Bergapten) was kindly supplied by Memphis Co., Cairo, Egypt. The drug was received in crystalline form, checked for purity by thin layer chromatography and gas-liquid chromatography and given to the volunteer patients in gelatine capsules (10 mg/capsule).

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TABLE 1. Scale for grading erythema and pigmentation

Scale	Degree of erythema	Scale	Degree of pigmentation
0	No erythema	0	No change in basic skin colour
1+	Minimal perceptible erythema	1+	Minimal perceptible tan
2+	Light pink erythema	2+	Light brown pigmentation
3+	Marked erythema, red in colour, but no oedema	3+	Moderate or medium brown pigmentation
4+	Fiery red erythema with oedema and tenderness	4+	Dark brown pigmentation
5+	Violaceous red erythema accompanied by marked oedema, blistering and tenderness	5+	Intense deep brown pigmentation

and 8-MOP (Oxsoralen; Gerot Pharmaceuticals, Vienna, Austria) respectively, 2 h prior to irradiation. To avoid reactions due to residual psoralen from foregoing tests, there was an interval of at least 2 days between each test. The protocol of the tests was identical with the one reported earlier for standard 8-MOP PUVA (Wolff *et al.*, 1977a). Six test fields measuring 2·5 cm in diameter were irradiated with increasing doses of UV-A, the rest of the body being covered. UV-A was delivered by a high energy output UV source (PUVA 500, 4000, 6000 irradiations units, Messrs. Waldmann, Schwenningen, FRG) radiating at a continuous spectrum between 320 and 390 nm (maximum at 365 nm). Energies ranged from 6·0 to 9·0 mW/cm^2 at a distance of 5–15 cm, as measured with a cosine corrected photometer (Ultraviolet Products, San Gabriel, California, U.S.A.). Readings of phototoxic reactions were made at 72 h on the basis of the criteria shown in Table 1. Since the common complexion of the population in our geographic area corresponds to that of type III light sensitivity (Wolff *et al.*, 1977a; Melski *et al.*, 1977), the majority of the patients were of skin type III.

Comparison of phototoxic reactions to 5-MOP and 8-MOP. Seven individuals were given identical doses of 5-MOP and 8-MOP (0·6–0·8 mg/kg body weight), which, on a mg/kg body weight basis, were identical with the 8-MOP doses used in standard 8-MOP PUVA. The test areas were irradiated with UV-A doses of 1·5, 2, 3, 5, 7, 9 J/cm^2, according to the testing schedule for skin types III/IV (Wolff *et al.*, 1977a).

Comparison of phototoxic reactions using various doses of UV-A and 5-MOP. Tests were performed on four individuals. Three different combinations of 5-MOP and light were investigated.

(1) 5-MOP was given in doses of 0·6–0·8 mg/kg; UV-A doses ranged from 1·5 to 9·0 J/cm^2. This corresponded to the doses of drug and light usually employed in phototoxicity testing for 8-MOP PUVA.

(2) 5-MOP was given in doses of 0·6–0·8 mg/kg, but the UV-A doses ranged from 3·0 to 18·0 J/cm^2. In this approach the 5-MOP dose, on a mg/kg basis, was kept at the 8-MOP level of 8-MOP PUVA but the UV-A energy was increased.

(3) The 5-MOP doses were 1·2–1·6 mg/kg; the UV-A doses ranged from 1·5 to 9·0 J/cm^2. Here, the UV-A doses were kept at the 8-MOP PUVA level whereas the drug dose was doubled.

Treatment of psoriasis

Standard 8-MOP PUVA treatment. This treatment was performed according to standard procedures as outlined previously (Wolff *et al.*, 1977a). Fifty-nine patients with generalized chronic plaque type psoriasis were treated* and the results were compared with the results of the 5-MOP PUVA group.

5-MOP PUVA treatment (high dose regimen). The nineteen patients with generalized plaque type psoriasis comprising this group were given 5-MOP PUVA therapy throughout the period of observation. The patients were randomly selected and informed consent was obtained†.

Two hours after the oral administration of 5-MOP at a dose of 1·2–1·6 mg/kg body weight, the patients were exposed to the UV-A light source. The dosimetry of UV-A for the initial dose was based on the results of foregoing phototoxicity tests in which the minimal phototoxic dose (MPD) had been determined, as described for standard 8-MOP PUVA (Wolff *et al.*, 1976). Since minor phototoxic reactions to 5-MOP often manifest with pigmentation without foregoing erythema, a barely perceptible erythema *or* pigmentation at the test site was considered to represent the MPD. Readings were made at 72 h. The MPDs and thus initial doses for therapy ranged from 4–14 J/cm^2. Treatments were given four times a week until complete clearing was achieved. Rapid tanning allowed UV-A dose increments ranging up to 6·0 J/cm^2 per increment, which were well tolerated by the patients.

After clearing three patients were entered into a maintenance therapy regimen, employing 5-MOP as outlined for 8-MOP PUVA (Wolff *et al.*, 1976). All other patients were subjected to standard 8-MOP PUVA maintenance treatment.

Paired comparison study 5-MOP vs. 8-MOP. Six patients were treated with 5-MOP PUVA on one half of the body, every other day. During irradiation the other half of the body was shielded by sheets; conversely, this side of the body was treated with 8-MOP PUVA on the following day and the 5-MOP PUVA side was shielded. For 8-MOP PUVA the patients received 0·6–0·8 mg 8-MOP and UV-A doses were given according to the results of 8-MOP phototoxicity testing preceding the treatment; initial doses ranged from 1·0 to 5·0 J/cm^2 and increments of the UV-A dose were made as described for standard 8-MOP PUVA (Wolff *et al.*, 1977a). For 5-MOP treatment the 5-MOP dose was 1·2–1·6 mg/kg and the UV-A doses were determined according to the results of 5-MOP phototoxicity testing. They ranged from 4·0 to 14·0 J/cm^2 for the initial treatment and increments were performed as described for the high dose group. Absence of scaling and flattening of psoriatic lesions to non-palpability on one side was considered the end point of the paired comparison.

Paired comparison study 8-MOP vs. 8-MOP+5-MOP. The rationale of this study was to determine whether 8-MOP and 5-MOP can be combined to increase the efficacy of PUVA without a concomitant increase of side-effects (erythema). Thirteen patients were treated. The technical details were identical to those in the previous section. For 8-MOP PUVA the patients received 0·6–0·8 mg 8-MOP and UV-A doses as in standard treatment to one side of the body. For the 8-MOP plus 5-MOP treatment, which was given to the other side of the body, 5-MOP and 8-MOP were simultaneously administered at a dose of 1·2–1·6 mg/kg of 5-MOP and 0·6–0·8 mg/kg of 8-MOP, 2 h before irradiation; the UV-A doses given had been determined by a combined 5-MOP+8-MOP phototoxicity testing. Absence of scaliness and flattening of lesions to non-palpability on either side was considered the end point of this study.

Low dose 5-MOP PUVA treatment. These regimens were employed to determine whether clinically recognizable phototoxic reactions are at all necessary in 5-MOP PUVA treatment.

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* This group is identical to the control group in another study (Fritsch *et al.*, 1978a).

† Informed consent was also obtained from all other patients and volunteers who received 5-MOP.

Group A. Nine patients were phototoxicity tested as described for the high dose group, employing 1·2–1·6 mg 5-MOP/kg body weight. After determination of the MPD, treatment was started with 0·6–0·8 mg 5-MOP/kg body weight employing only half the MPD of UV-A (i.e. when testing had revealed that with 1·2–1·6 mg/kg 5-MOP the MPD was 10 J/cm^2, the patient received only 0·6–0·8 mg 5-MOP and 5 J/cm^2 for the first treatment). Four such treatments were given per week at weekly intervals; the UV-A dose was increased by 20% whereas the 5-MOP dose was kept constant at 0·6–0·8 mg/kg and this was continued until the patient was 100% clear and treatment was interrupted.

Group B. Five patients were phototoxicity tested and treated with initial doses of drug and light as in group A. However, dose increments of UV-A in subsequent treatments were performed arbitrarily, according to the response of the patient; dose increments were thus higher and more frequent in this group than in group A.

Laboratory examinations

All patients were subjected to physical and ophthalmological (slit lamp and fundoscopy) examination. The following laboratory studies were performed: erythrocyte sedimentation rate, complete blood count, blood glucose, serum glutamic oxalacetic and pyruvic acid, lactic acid dehydrogenase, alkaline phosphatase, blood urea nitrogen, uric acid and serum creatinine. Urine analyses were also performed.

RESULTS

Phototoxicity tests

Comparison of phototoxic reactions due to 5-MOP and 8-MOP. When equal doses (0·6–0·8 mg/kg) of 5-MOP and 8-MOP were employed the 5-MOP test sites showed neither erythema nor pigmentation after 72 h in six of seven patients, whereas with 8-MOP all but two patients reacted (Table 2).

Comparison of phototoxic reactions with various doses of 5-MOP and UV-A. These results are shown in Table 3. When the low dose of 5-MOP (0·6–0·8 mg/kg) was employed no erythema and only a

TABLE 2. Erythema (E) and pigmentation (P) readings after 72 h

	5-MOP (0·6–0·8 mg/kg)							8-MOP (0·6–0·8 mg/kg)						
Patient/skin type	J/cm^2	9	7	5	3	2	1	J/cm^2	9	7	5	3	2	1
1/III	E	±	–	–	–	–	–	E	++	++	+	+	±	±
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
2/IV	E	–	–	–	–	–	–	E	±	±	–	–	–	–
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
3/III	E	–	–	–	–	–	–	E	–	–	–	–	–	–
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
4/II	E	–	–	–	–	–	–	E	+++	++	++	+	+	±
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
5/III	E	–	–	–	–	–	–	E	–	–	–	–	–	–
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
6/III	E	–	–	–	–	–	–	E	+	+	+	+	±	±
	P	–	–	–	–	–	–	P	–	–	–	–	–	–
7/III	E	–	–	–	–	–	–	E	+	+	+	±	–	–
	P	–	–	–	–	–	–	P	–	–	–	–	–	–

TABLE 3. Erythema (E) and pigmentation (P) readings after 72 h

5-MOP (mg/kg)		0·6–0·8						1·2–1·6						1·2–1·6					
UV-A (J/cm²)		18	14	10	6	4	3	9	7	5	3	2	1	18	14	10	6	4	3
Patient/skin type																			
1/III	E	–	–	–	–	–	–	±	±	–	–	–	–	+	+	±	–	–	–
	P	±	±	±	–	–	–	–	–	–	–	–	–	±	±	±	–	–	–
2/III	E	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	P	–	–	–	–	–	–	–	–	–	–	–	–	±	±	–	–	–	–
3/III	E	–	–	–	–	–	–	–	–	–	–	–	–	+	+	±	–	–	–
	P	±	±	±	–	–	–	±	±	±	–	–	–	+	±	±	±	±	±
4/III	E	–	–	–	–	–	–	±	±	–	–	–	–	+	+	±	±	±	–
	P	±	±	–	–	–	–	–	–	–	–	–	–	±	±	±	±	–	–

questionable pigmentation was produced even with UV-A doses as high as 18 J/cm². Erythema and/or pigmentation did, however, result when a higher 5-MOP dose regimen was employed. As is shown in Table 3, there was a good correlation between these reactions and the doses of 5-MOP and UV-A employed. However, the erythemas were only moderate and even increments of both light and drug never resulted in more than a 1+ erythema within the test fields.

Treatment of psoriasis

8-MOP PUVA. In all fifty-nine patients complete clearing was achieved after 14±8 irradiations within a period of 28±15 days. No treatment failure occurred in this group (Table 4).

High dose 5-MOP PUVA treatment. Using 5-MOP at a dose of 1·2–1·6 mg/kg the number of treatments and of days required for clearing was about one third less than with standard 8-MOP PUVA, whereas the UV-A energy requirements were comparable for both regimens. The '*t*'-test for means of random distribution showed the differences in the number of treatments and duration of treatment to be significant ($P<0·02$ for both) (Table 4). All nineteen patients were cleared. Pigmentation developed considerably faster than in 8-MOP therapy, allowing dose increments ranging up to 6·0 J/cm². As expected from the results of the phototoxicity tests even such high dose increments did not lead to a notable erythematous reaction, except in one patient (see below).

TABLE 4. 5-MOP (high dose) vs. 8-MOP/PUVA treatment

	5-MOP/PUVA	8-MOP/PUVA
Number of patients	19	59
Patients completely cleared	19	59
Days required for clearing*	17·1±5·6	27·8±15·2
Exposures required for clearing*	9·0±3·4	14·3±7·8
UV-A dose (J/cm²) required for clearing†	105·3±70·9	93·2±80·6

* $P<0·02$.
† Difference not significant.

TABLE 5. 8-MOP vs. 5-MOP (high dose)+8-MOPPUVA treatment

	Number of exposures (days) required for clearing		Dose required for clearing (J/cm²)	
	8-MOP	5-MOP+8-MOP	8-MOP	5-MOP+8-MOP
Patients (n = 13)	7·1±2·5 (18·7±5·0)	7·2±2·4 (18·4±5·0)	26·3±13·6	25·4±15·6

Maintenance therapy. After complete clearing of psoriatic lesions a maintenance treatment with 5-MOP was started in three patients according to the guide lines elaborated for standard 8-MOP PUVA. Preliminary results indicate that the maintenance therapy with 5-MOP can keep the patients in remission up to 10 months.

Paired comparison study of 5-MOP PUVA vs. 8-MOP PUVA. In this paired comparison treatment the end point of the study, i.e. complete flattening of psoriatic lesions, was reached earlier on the 5-MOP treated side in all patients. The mean number of irradiations required for this improvement was 6·2±1·1. At this time the 8-MOP PUVA side was also improved, but less than the 5-MOP PUVA-treated side of the body. The paired comparison study thus verified the results obtained with the high dose 5-MOP regimen.

Paired comparison study of 8-MOP+5-MOP PUVA. In this study both sides cleared simultaneously and there was no significant difference in the number of exposures and days and the amount of UV-A energy required for clearing (Table 5). Since erythema due to 8-MOP UV-A develops more readily and with lower doses of UV-A energy than erythema due to 5-MOP UV-A, and since erythema is the limiting factor in PUVA therapy—both with regard to phototoxicity testing and treatment—it may be that the therapeutic effect observed on the 5-MOP+8-MOP PUVA-treated side of these patients was due to 8-MOP PUVA alone; possibly, not enough UV-A energy was delivered to the 5-MOP+8-MOP side to elicit the 5-MOP UV-A effect observed in the patients who had received 5-MOP alone.

Low dose 5-MOP PUVA treatment. Group A. Six out of nine patients in this group were cleared although both the 5-MOP and UV-A dose were only half of that required for a clinically visible phototoxic reaction and dose increments were only 20% of the initial dose and were performed only once a week. Twelve treatments and 21 days were necessary to obtain this result (Table 6). The involution of psoriatic lesions was identical to that observed during standard PUVA treatment; erythema never developed. However, three of the original nine patients did not clear completely after 3 weeks

TABLE 6. Low dose 5-MOP PUVA treatment (group A)

Number of patients	9
Patients completely cleared	6
Days required for clearing	20·5±7·9
Exposures required for clearing	12·0±4·6
UV-A dose (J/cm²) required for clearing	105·8±59·6

TABLE 7. Low dose 5-MOP PUVA treatment (group B)

Number of patients	5
Patients completely cleared	5
Days required for clearing	22·0 × 8·6
Exposures required for clearing	11 8 ± 5·4
UV-A dose (J/cm²) required for clearing	80 5 ± 29 2

and treatment was thus discontinued at this time. Two of them were 90% and one only 30% improved. *Group B.* All five patients cleared completely after 22 days requiring twelve exposures (mean) (Table 7). There were considerable dose increments in this group, the final UV-A dose often being three times the dose given for the first treatment. Erythema did not develop but pigmentation was considerable with this regimen.

Side-effects

Table 8 summarizes the side-effects observed in the 5-MOP PUVA and 8-MOP PUVA treatment groups. In 5-MOP PUVA a localized + + + erythema was observed in only one patient (skin type I) in a previously unexposed area (buttocks). There was no nausea in the 5-MOP group and neither was pruritus observed in these patients. An unexplained phenomenon was the bleaching of hair in two patients; this, however, proved reversible, when the patients were switched to 8-MOP during maintenance therapy. Laboratory data showed no pathological changes as compared with the initial examination. In a few patients, initially abnormal liver profiles returned to normal or improved during therapy, possibly because patients refrained from alcohol consumption during therapy. The side-effects observed in the 8-MOP group were within the range of previous series of patients (Wolff *et al.*, 1977b).

Pigmentation

There was rapid and pronounced tanning in all patients treated with 5-MOP PUVA. This tan was uniform and developed much faster than in the patients of the 8-MOP PUVA control group or on the

TABLE 8. Side effects

	Number of patients	
	5-MOP	8-MOP
Localized + + + erythema (previously unexposed areas)	1	4
Nausea	0	4
Pruritus	0	12
Kobner phenomenon	0	0
Photo-onycholysis	0	3
Bleaching of hair	2	0
Abnormal laboratory tests (as compared with initial examination)	0*	0*

* In a few patients abnormal liver profiles improved or returned to normal during therapy.

8-MOP PUVA-treated sides in the paired comparison studies. However, after clearing 5-MOP and 8-MOP PUVA-treated patients exhibited the same degree of tanning. The patients on the low dose 5-MOP PUVA regimen also tanned considerably although pigmentation developed slower than in the 'regular' 5-MOP PUVA group. Towards the end of the clearing phase, the degree of pigmentation was roughly the same in all 5-MOP patient groups. We had the impression that 5-MOP PUVA-induced tanning persisted longer than a regular suntan but measurements to quantify this observation were not done.

DISCUSSION

Attempts at improving photochemotherapy should aim at a better standardization of the existing regimen, an augmentation of its efficacy and a reduction of discomforting side-effects, and a reduction of the probability of long term hazards. Specifically, this would include a reduction of the duration and frequency of treatments during the clearing and maintenance phases to gain even better patient acceptance; a reduction of the amount of UV-A energy required for the treatments to avoid possible cumulative skin damage related to either the UV-A energy alone or the phototoxic reactions; and a prevention or reduction of undesired side effects caused by 8-MOP alone, such as nausea, or by the phototoxic reaction, such as erythema and pruritus which mainly result from UV-A overdosage (Wolff *et al.*, 1975, 1976).

Approaches that could lead to an improvement of PUVA therapy include the improvement of the drug preparation to provide better absorption and/or better tolerance by the patient, or the combination of PUVA with other topical or systemic drugs or regimens. PUVA has been used in combination with topical corticosteroids and tar (Schmoll, Henseler & Christophers, 1978; Morison, Parrish & Fitzpatrick, 1978), but the most effective modification of the PUVA regimen reported so far consists of the combination of PUVA with an oral aromatic retinoid (Fritsch *et al.*, 1978a, b). This regimen has been found to reduce dramatically the total UV-A energy required for clearing psoriasis and to enhance the therapeutic effectiveness of PUVA by 50%. Moreover, it renders patients who are PUVA-treatment failures responsive to this therapy (Fritsch *et al.*, 1978a).

The employment of a new or different photosensitizing drug in PUVA therapy is another alternative in attempts to improve PUVA and this was the approach attempted in this study. 5-MOP indeed possesses photosensitizing capacities but when administered orally much higher doses of either drug or UV-A energy are required to induce erythema than in 8-MOP UV-A phototoxicity reactions. The phototoxicity tests with 5-MOP revealed that erythematous reactions can hardly be produced with doses of drug and UV-A which are equivalent to those employed in 8-MOP PUVA (Wolff *et al.*, 1977a); only by raising the 5-MOP dose to twice that of the usual 8-MOP dose and by irradiating with high levels of UV-A energy was it possible to elicit erythema. On the other hand, 5-MOP induces pigmentation more rapidly than 8-MOP and pigmentation ensues without foregoing erythema, the advantage of which is obvious: the effect of 5-MOP UV-A on the skin can be assessed clinically without erythematous responses.

In this study, 5-MOP proved an excellent drug for photochemotherapy. Using a high dose regimen, 5 MOP PUVA was more effective than 8-MOP PUVA. It produced less erythema and pruritus and was not accompanied by nausea—a troublesome side-effect of 8-MOP PUVA—even with the large doses of 5-MOP employed. Though significant in our series, the better response of psoriatics to 5-MOP PUVA than to 8-MOP PUVA will require substantiation in a larger series of patients, but the lower incidence of side-effects does seem to indicate that the employment of 5-MOP is preferable to 8-MOP in PUVA treatment. However, although the total UV-A energy required for clearing was roughly the same in the 5-MOP PUVA and 8-MOP PUVA regimens, the relative UV-A doses for the individual 5-MOP PUVA treatments were higher and it is presently not clear whether this may have a bearing on the potential long term hazards possibly inherent in long term PUVA therapy (Fitzpatrick *et al.*, 1977).

The mechanism by which 5-MOP PUVA leads to an involution of psoriatic lesions is unknown. 5-MOP is known to interact with DNA under the influence of UV-A (Rodighiero & Dall'Acqua, 1976) and by analogy to what is believed to occur in 8-MOP PUVA at the cellular level (Walter *et al.*, 1973; Fritsch *et al.*, 1978c), it may be postulated that 5-MOP DNA photoadducts and crosslinks reduce the rapid replication of epidermal cells in psoriatic skin. However, the different furocoumarins have varying abilities to form links with pyrimidine bases and proteins owing to differences in the geometric structure of the psoralen derivatives, and the interactions of 5-MOP and 8-MOP with UV-A therefore differ in several respects (Rodighiero & Dall'Acqua, 1976). Since this suggested the possibility that 5-MOP and 8-MOP PUVA could act synergistically or in a potentiating manner in the treatment of psoriasis a group of patients were given full doses of 8-MOP and 5-MOP simultaneously. However, in this series the therapeutic effect was comparable to the effect of 8-MOP PUVA alone. As mentioned above, one of several possible explanations for this phenomenon might be that the 8-MOP erythema prevents the use of higher UV-A doses which would be required for adequate photochemical reactions to occur with 5-MOP.

In 8-MOP PUVA therapy the limiting factor for increments of the UV-A dose during the initial phases of the treatment is erythema. Dose increments thus have to be determined carefully and usually should not exceed 2·0 J/cm^2 (Wolff *et al.*, 1977a). By contrast, in the 5-MOP PUVA patients, the low propensity to develop erythema and the rapidly developing tan permitted more aggressive UV-A dose increments which, in one patient, reached levels as high as 6·0 J/cm^2. This contributed to the observed shortening of the clearing phase—as compared with the 8-MOP PUVA patients—but also resulted in a total UV-A dose which approximated that given to the 8-MOP patients. The fact that the excellent therapeutic results of 5-MOP PUVA were achieved without the development of erythema in most patients indicates that erythema is only one aspect of psoralen UV-A phototoxicity and apparently is not a prerequisite for the beneficial therapeutic effect of this principle.

Our low dose 5-MOP PUVA regimens (groups A and B) provide unequivocal evidence to support this conclusion. An involution of psoriatic plaques and clearing of the patients was achieved with doses of 5-MOP and UV-A which did not and could not possibly have led to erythema and, although in group A the response of the patients was not as complete as with a standard 8-MOP PUVA, high dose 5-MOP PUVA and low dose 5-MOP PUVA (group B) regimens, the observed results are of considerable significance. 5-MOP PUVA treatment of psoriasis can be performed under conditions which, with regard to acute PUVA side-effects, can be considered absolutely safe.

Considering the fact that, with the doses employed, 5-MOP does not cause nausea and that 5-MOP PUVA is not followed by pruritus, this treatment therefore presently appears an ideal therapy for psoriasis. It will have to be determined whether 5-MOP produces more or less photoadducts and crosslinks upon irradiation with UV-A than 8-MOP. Should the latter be the case, 5-MOP PUVA could also be considered less hazardous with regard to long term sequelae. However, a word of caution appears appropriate in this context. In view of long term chronic effects, it is at present difficult to understand that a photochemical reaction which leads to the disappearance of psoriatic plaques and produces pigmentation without erythema should be any less serious, on the molecular level, than a reaction in which erythema occurs.

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Photochemotherapy (PUVA) of psoriasis using 3-carbethoxypsoralen, a non-carcinogenic compound in mice

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SUMMARY

The carcinogenic risk of photochemotherapy (PUVA) with bi-functional furocoumarins such as 8-methoxypsoralen (8-MOP) which form cross-links in cellular DNA has initiated a search for active but less hazardous psoralens. A new compound, 3-carbethoxypsoralen (3-CPs), studied in the yeast *Saccharomyces cerevisiae* (eukaryote), has been shown to be very photoactive on DNA and to form only mono-additions to DNA. These lesions appear to be more easily repaired than the cross-links induced by 8-MOP. 3-CPs produces less nuclear genetic events such as nuclear mutations and mitotic crossovers, but more cytoplasmic 'petite' mutations (damage to mitochondrial DNA) than 8-MOP. In mice it was demonstrated that after local or intra-peritoneal administration, in contrast to 8-MOP, 3-CPs is non-toxic, non-erythematogenic, and non-carcinogenic. A study of ten psoriatic patients has shown that local applications of 3-CPs plus UV-A exhibit about the same therapeutic activity for the clearing of psoriatic lesions as local treatment with 8-MOP plus UV-A, but without any localized hyperpigmentation.

Psoralens used in photochemotherapy of psoriasis exhibit two reactive sites, the double bonds 3–4 4′–5′ (see for example, 8-methoxypsoralen, Fig. 1).

Psoralens are able to bind covalently to pyrimidine bases in DNA under the action of UV-A, i.e. ultraviolet radiation of 320–380 nm. Bi-functional compounds like 8-methoxypsoralen (8-MOP) (Fig. 1) form mono- and bi-additions (cross-links) (see for review, Pathak, Kramer & Fitzpatrick, 1974). These compounds produce erythema and hyperpigmentation of the skin, and show a remarkable healing activity on psoriatic lesions (Melski *et al.*, 1977). However, in PUVA therapy they are used with some caution since they are not only highly mutagenic in various organisms (see for review,

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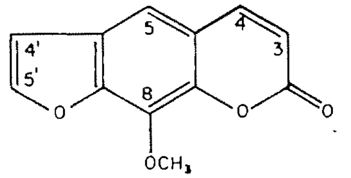

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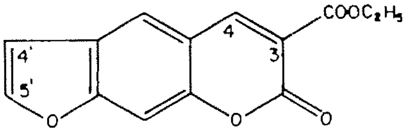

FIGURE 1. Molecular structure of the bi-functional furocoumarin 8-methoxypsoralen (8-MOP).

FIGURE 2. Molecular structure of the mono-functional furocoumarin 3-carbethoxypsoralen (3-CPs).

Scott, Pathak & Mohn, 1976) but are also carcinogenic in mice after cutaneous and intraperitoneal administration (Griffin, Hakim & Knox, 1958; Griffin, 1959; Hakim, Griffin & Knox, 1960; Grube, Lay & Fry, 1977).

Naturally occurring or synthetic psoralens do exist in which, either for steric reasons or due to a chemical substitution, only a single reaction site is available for the photoreaction. These so-called mono-functional furocoumarins are just capable of forming mono-additions with cellular DNA. They are hardly (or not at all) erythematogenic on the human skin, and for this reason, have been discarded from clinical tests as therapeutically inactive (Pathak *et al.*, 1974). However, some of them possess a considerable photo-affinity to DNA (Musajo *et al.*, 1974), and they are able to block DNA synthesis (Bordin *et al.*, 1976). These mono-additions are more easily removed by cellular repair mechanisms than the cross-links induced by the bi-functional agents (Chandra *et al.*, 1974; Bordin *et al.*, 1976; Averbeck, Moustacchi & Bisagni, 1978).

With regard to these facts it seemed legitimate to ask whether the therapeutic activity of psoralens depends on the type of lesions induced in DNA, the cross-links or the mono-additions, and whether these lesions have the same mutagenic and carcinogenic consequences. For example, would the mono-functional compounds be less mutagenic than the bi-functional agents? If so, one could hope that a reduced mutagenicity would be associated with a decreased carcinogenicity, whereas the therapeutic activity would still be important.

Thus, we studied different mono-functional derivatives of psoralen in order to search for a compound which still exhibits an appreciable photo-reactivity, but which could be expected to present a lower carcinogenic risk than the psoralens used in therapy at present. From a series of different furocoumarins tested on yeast, we selected the 3-carbethoxypsoralen (3-CPs), a synthetic compound (Queval & Bisagni, 1974), in which one reactive site of the psoralen molecule, the 3–4 reaction site, is occupied by a carbethoxy group (Fig. 2). Before showing the therapeutic results, we describe some of the experimental findings which demonstrated a remarkable photoactivity of this compound and the absence of carcinogenic side-effects.

Photosensitizing action of 3-CPs in yeast (Saccharomyces cerevisiae)

From its molecular structure (Fig. 2) a poor cross-linking activity of 3-CPs could be expected. Indeed, it was demonstrated that 3-CPs is unable to form cross-links with DNA, but is able to induce mono-additions (Averbeck *et al.*, 1978). The photo-affinity of 3-CPs to yeast DNA *in vivo* in the presence of UV-A was about sixteen fold higher than that of 8-MOP. In spite of this, 3-CPs was 5–6 times less active than 8-MOP in cell killing in yeast, suggesting that the photo-induced mono-additions are more efficiently repaired than the cross-links produced by 8-MOP. 3-CPs was very efficient in the induction of cytoplasmic 'petite' mutations which depend on the induction of lesions in mitochondrial DNA (Averbeck *et al.*, 1978; Averbeck & Moustacchi, 1979). 3-CPs shares with a number of

TABLE 1. Comparison between 3-carbethoxypsoralen (3-CPs) plus UV-A and 8-methoxypsoralen (8-MOP) plus UV-A in mice

	No.	Irradiation (J/m²)	Strain	No. of animals	Animals at risk	Animals with tumour (%)	Animals with a second tumour (%)
Topical applications	115 × 8-MOP*	$1{\cdot}68 \times 10^4$	XVIInc/Z	40	37	92	15
	106 × 3-CPs*	$1\ 68 \times 10^4$	XVIInc/Z	40	39†	0	0
	196 × 3-CPs*	$3{\cdot}36 \times 10^4$	XVIInc/Z	40	37†	0	0
Intraperitoneal injections	36 × 0 4 mg/8-MOP/animal	$1{\cdot}68 \times 10^4$	Swiss	40	37	90	5
	36 × 0·4 mg/3-CPs/animal	$1{\cdot}68 \times 10^4$	XVIInc/Z	40	38‡	0	0
Controls	106 × —	$1{\cdot}68 \times 10^4$	XVIInc/Z	20	17‡	0	0
	106 × 3-CPs*	—	XVIInc/Z	20	19‡	0	0

* 15 μg/cm² in acetone solution.

† Number of mice alive at the age of 22 months.

‡ Number of mice alive when the first carcinoma was observed in each group.

other antipsoriatic drugs the capacity to induce cytoplasmic 'petite' mutations in yeast (Swanbeck & Thyresson, 1974). Using mutants of yeast deficient in the repair of induced nuclear lesions, it was shown that genetically controlled repair processes, such as excision-resynthesis and a recombinational type of repair, contribute to the overall repair of 3-CPs induced lesions in wild type cells (Averbeck *et al.*, 1978).

For the induction of nuclear mutations 3-CPs was clearly less effective than 8-MOP (Averbeck *et al.*, 1978; Averbeck & Moustacchi, 1979). The same was true for the induction of mitotic crossovers as a function of survival (Averbeck & Moustacchi, 1979; Aberveck *et al.*, 1978). The induction of mitotic gene conversions per viable cell was about the same for 3-CPs and 8-MOP (Averbeck & Moustacchi, 1979). The low mutagenicity of 3-CPs in comparison to 8-MOP suggested the possibility of a lower carcinogenic activity.

Effects of 3-CPs plus UV-A in mice

Forty mice (males and females) aged 12–14 weeks, belonging to the homozygous strain XVIIn c/Z, were used for each type of experiment. Forty microlitres of an acetone solution of 3-CPs or 8-MOP (approx. 15 μg/cm^2) were applied to each ear 15 min before irradiation. To prevent the animals from shielding one another and from licking each other's ears before and during irradiation, the mice were immobilized in compartments of a specially designed square cage covered with a fine mesh grating. The distribution of the mice in the different compartments of the cage varied randomly from treatment to treatment.

Irradiations were performed by means of a device consisting of four Philips 125 lamps with Edison caps and a running water filter constructed with two Pyrex glass slides. The Philips HPW 125 lamps emit light mostly in the 365 nm wavelength region. The Pyrex glass filter stops all light under 340 nm and the water filter allows irradiation at room temperature. The dose rate for 365 nm from this source, at the level of the mice ears, was 28 J m^{-2} s^{-1}.

The animals were treated five times a week, all being exposed to the same irradiation dose: $1{\cdot}68 \times 10^4$ J m^{-2} (10 min every time). This schedule was chosen because it is close to that used for human treatments (Melski *et al.*, 1977). The animals receiving psoralen by intraperitoneal injections were studied under the same conditions as by Griffin *et al.* (1958) that is thirty-six injections of 0·4 mg of product in olive oil solution followed 60 min later by UV-A exposure to $1{\cdot}68 \times 10^4$ J m^{-2}. The animals were maintained in safety-lighted rooms, received autoclave sterilized complete food and Millipore-filtered water *ad libitum*. They were examined periodically and any modification was recorded on an individual card. All animals were autopsied and the lesions examined histologically.

Table 1 shows that 3-CPs is not carcinogenic for mouse skin, either by topical application or when administered by intraperitoneal injections. One hundred and ninety-six applications, representing about twice the dose resulting in nearly 90% tumours with 8-MOP, did not induce any detectable skin lesion. Thirty-six intraperitoneal injections of 0·4 mg/mouse (20 mg/kg) of 3-CPs followed by UV-A irradiation resulted in no sign of general toxicity, either acute or delayed. Using ^{14}C labelled 3-CPs we have shown that this compound rapidly penetrates into the mouse epidermis (data not shown).

Experiments with intraperitoneal injections of 8-MOP, performed under conditions close to those of Griffin *et al.* (1958), confirmed the latter's results. Ninety percent of Swiss mice developed tumours (mostly sarcomas) on the ears but also on other hairless areas (paws). Most of the animals became blind. Chronic topical application of 8-MOP (115 treatments) induced 90% epidermal tumours—in the majority squamous cell carcinomas, more or less well differentiated. Metastases in cervical lymph nodes were frequent. These results were close to those obtained by previous workers (Griffin *et al.*, 1958; Griffin, 1959; Hakim *et al.*, 1960; Grube *et al.*, 1977).

Taking these results on the photoactivity of 3-CPs together with those on the absence of carcinogenic

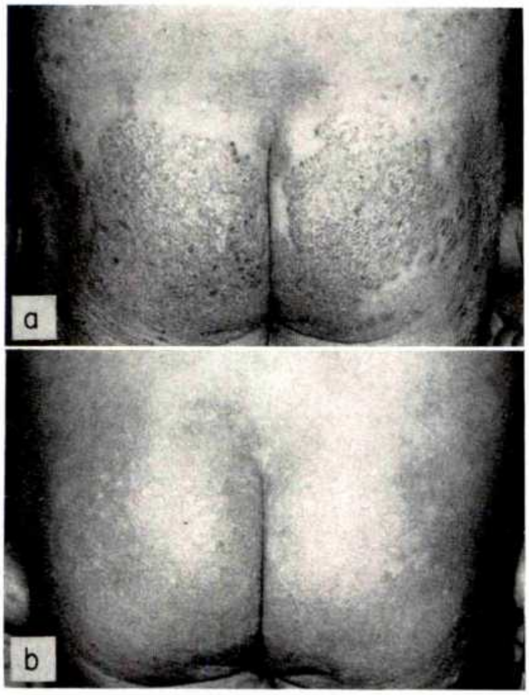

FIGURE 3. Clearing of psoriatic lesions (Case 5). (a) Before, (b) after treatment with 3-carbethoxypsoralen (3-CPs) and UV-A (excellent result).

and toxic effects of 3-CPs (results obtained in 1977), and with regard to the known risks linked to photochemotherapy with 8-MOP, we considered ourselves justified in starting a small scale clinical and therapeutic trial on ten patients suffering from limited psoriasis, using locally administered 3-CPs and UV-A.

MATERIALS AND METHODS

3-CPs was synthesized according to Queval & Bisagni (1974). Its purity was controlled by the melting point, nuclear magnetic resonance spectra, the UV-absorption spectrum and the Rf in thin layer chromatography (Averbeck *et al.*, 1978). Since it was shown earlier that the penetration of 8-MOP was greatly favoured by using lanolin (Kammerau *et al.*, 1976), we also used this.

A cream containing 2% of 3-CPs was prepared by incorporating finely ground 3-CPs into lanolin (Hydrocerine Roc) heated to 70°C. In view of the differences in photobiological activity (Averbeck *et al.*, 1978), the concentration of 3-CPs chosen was higher than that used for 8-MOP treatments by other authors (0·15% to 1%) (Walter & Voorhees, 1973; Weber, 1974; Schaefer *et al.*, 1976; Petrozzi & Kligman, 1977). As a preservative we added 0·4% parahydroxymethylbenzoate. The cream (2% 3-CPs) was applied to the skin lesions 2 h before irradiation, as in PUVA therapy with 8-MOP.

The irradiation chambers were the Waldmann 4000 and 6000 equipped with Sylvania FR 74T12 tubes. The same lamps as used with 8-MOP could be used since 3-CPs absorbs in about the same range of wavelengths (Aberbeck *et al.*, 1978). The starting treatment involved four sessions per week.

TABLE 2. Clinical observations on ten psoriatic patients after treatment with 3-carbethoxypsoralen (3-CPS) plus UV-A

Case		Results*	Number of irradiations	Total dose (J/cm^2)	Effect of photosensitization†	Köbner reaction	Reappearance of lesions at treatment maintenance	Localized hyperpigmentation
A.L.	1	++	19	162	o	o	+	o
C.H.	2	+	10	50	+++	o	—	o
F.L.	3	+++	20	300	±	o	o	o
D.E.	4	++	17	255	±	o	+	o
R.E.	5	+++	48	720	o	o	+	o
D.U.	6	+++	17	190	o	o	+	o
G.U.	7	++	8	120	+++	+	—	o
S.T.C.	8	+++	47	471	++	+	o	o
de F.	9	o	17	170	o	o	—	o
B.O.	10	+	15	210	+++	+	—	o

* Therapeutic results:
o = failure.
+ = insufficient.
++ = medium.
+++ = excellent.

† Photosensitization:
o = absent.
+ = slight.
++ = forced to decrease doses.
+++ = forced to stop treatment.

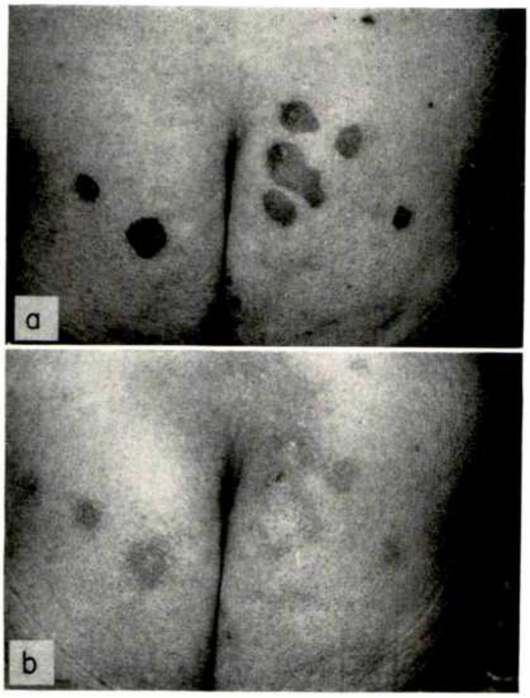

FIGURE 4. Clearing of psoriatic lesions (Case 1). (a) Before, (b) after treatment with 3-carbethoxypsoralen (3-CPs) and UVA (medium result).

The irradiation dose was 15 J cm^{-2} per session corresponding to the energy output at wavelengths between 347 and 383 nm measured by the Waldmann photometer incorporated in the PUVA 6000 unit. The irradiation doses were reduced in case of secondary effects. The maintenance treatment, which involved one session per week, was started when sufficient improvement had been achieved (Fig. 3a and b).

We treated ten psoriatic patients in whom the disease had been present for an average of 11·9 years and in which the psoriatic patches covered about 25% of the body surface on average.

RESULTS

The therapeutic results using 3-CPs are summarized in Table 2. We obtained four excellent results (Cases 3, 5, 6 and 8) after an average of thirty-three sessions and with a mean total UV-A dose of 417 J cm^{-2}. These results include a patient with extensive psoriasis (Case 5, Fig. 3a and b) who was cleared after forty-eight sessions, and another patient (Case 8) who was treated for forty-seven sessions. This latter patient suffered from a transient photosensitization plus Köbner phenomenon during the treatment period which retarded the clearing of the psoriatic patches.

Three patients (Cases 1, 4 and 7) had good results. Case 1 (Fig. 4a and b) had a partial amelioration; Case 4 a rapid amelioration but a relapse during the initial treatment. Case 7 showed a rapid improvement followed by a strong photosensitization which compelled us to stop the treatment.

Amongst the unsuccessful cases (2, 9 and 10), one was classified as a complete failure (Case 9)

TABLE 3. Comparison of the therapeutic efficacy of mono-functional furocoumarin 3-carbethoxypsoralen (3-CPs) plus UV-A with that of bi-functional furocoumarin 8-methoxypsoralen (8-MOP) plus UV-A

Study		Dubertret	Petrozzi & Kligman (1977)	Schaeffer *et al.* (1976)	Weber (1974)	Walter & Voorhees (1973)
Number of patients		16	30	62	74	11
Psoralens used		3-CPs (3%)	8-MOP (1%)	8-MOP (0·15%)	8-MOP (0·15%)	8-MOP (0·025%, 0·25%)
Excellent results	Number	4	12	17	34	2
	Percentage	50	40	28	46	19
	Average number of irradiations needed	30	18·16	9·7	27	—
Medium results	Number	3	9	30	23	6
	Percentage	20	30	48	31	54
	Average number of irradiations needed	12·5	18·55	10	30	—
Insufficient results	Number	3	9	15	17	3
	Percentage	30	30	24	23	27
	Average number of irradiations needed	14	16·5	10	17	—
Secondary effects	Kobner	3	2	1	3	3
	Erythema	6	frequent	21	frequent	frequent

since there was no response to the therapy with 3-CPs plus UV-A. In the two other cases (2 and 10) photosensitization forced us to interrupt the treatment before any improvement could be seen.

Side-effects

The photosensitization observed in six cases appeared after 14 days of treatment, i.e. after eight sessions. In two patients this effect was quite slight, in one of a transient character, and in three patients it led us to stop the treatment. Control tests with irradiation alone, with 3-CPs cream alone or with the lanolin ointment, made on healthy skin of the patients with and without irradiation showed no sensitization effect.

In three cases (7, 8 and 10) we observed a Kobner phenomenon. In four patients, the reappearance of psoriatic lesions was observed during the maintenance treatment. In two cases (1 and 6) continuing maintenance treatments was sufficient for a further improvement. In the other two patients treatment was continued with 8-MOP taken orally, but again lesions reappeared with one session per week.

In contrast to treatments using topical 8-MOP+UV-A, topical 3-CPs+UV-A did not induce localized hyperpigmentation.

DISCUSSION

This preliminary study on ten psoriatic patients clearly demonstrates that in some patients (four out of ten) good therapeutic results were obtained using the monofunctional furocoumarin, 3-CPs. It is interesting to compare the therapeutic efficacy of 3-CPs after local treatment with that obtained after local treatment with 8-MOP, as reported by four different teams (Walter & Voorhees, 1973; Weber, 1974; Schaefer *et al.*, 1976; Petrozzi & Kligman, 1977) (see Table 3).

Despite the different methods used, the results obtained with local 8-MOP are quite comparable. One group reported less excellent therapeutic results (Schaefer *et al.*, 1976); however, this is probably due to the fact that treatment was very short. The high concentrations of 8-MOP (1%) used by Petrozzi & Kligman (1977) did not produce a better therapeutic effect since the phototoxic side-effects increased. The results of Walter & Voorhees (1973) are difficult to interpret because they used different concentrations of 8-MOP.

Our preliminary study on ten psoriatic patients using 3-CPs plus UV-A shows therapeutic results comparable to those obtained by local treatment with 8-MOP (Table 3). It can be seen that the therapeutic improvement after 3-CPs is less rapid than with 8-MOP and a bigger total UV-A dose is needed with 3-CPs than with 8-MOP.

These results may be somewhat comparable to those obtained by Thune & Volden (1977) using low dose oral 8-MOP PUVA therapy. They observed a delay in the clearing of psoriatic lesions with low dose therapy.

The therapeutic improvement with 3-CPs was not due to the high doses of UV-A, since treatments with UV-A alone at these doses have been shown to be without effect on psoriasis (Walter & Voorhees, 1973; Fischer, 1976).

The localized hyperpigmentation that is constantly observed after local treatment with the bifunctional furocoumarin 8-MOP was absent after local treatment with the mono-functional furocoumarin 3-CPs. Thus, it is likely that the pigmentation produced by 8-MOP is not due to the formation of mono-additions in cellular DNA but is a consequence of the induction of DNA cross-links.

The erythema and pruritus sometimes observed after local treatments with 3-CPs were probably a consequence of a phototoxic phenomenon. The absence of such an effect when using the 3-CPs cream alone (without UV-A) and the decrease of these effects when using smaller doses of UV-A is in agreement with such an interpretation.

We noticed that photosensitization is not necessary for achieving a good therapeutic result, since two very good results (Cases 5 and 6) and one medium result (Case 1) were obtained without producing any erythema.

When photosensitization occurred it was first observed 14 days after starting the treatment and after at least eight treatment sessions. No hyperpigmentation was seen subsequently. These effects strikingly differ from the phototoxic effects produced after treatments with 8-MOP, where the phototoxic reaction occurs 72 h after a single treatment, leading afterwards to hyperpigmentation (Weber, 1974). It is possible, however, that photosensitization somewhat similar to that observed after treatment with 3-CPs may be produced also by the bi-functional furocoumarins but that it is masked by the early phototoxic reactions. Other possible ways to explain the photosensitization by 3-CPs may relate to the 3-CPs itself or the ointment used for the preparation of the 3-CPs cream. Photoproducts of 3-CPs (as seen by fluorescence techniques (Vigny, Gaboriau & Duquesne, personal communication)) formed after exposure to UV-A or the photo-reactions of 3-CPs itself could be phototoxic. Alternatively, an esterification could occur with the ointment involving the carbethoxy group of the 3-CPs molecule and the alcoholic group of the lanolin. Such a reaction could be favoured by UV-A radiation, and erythematogenic photo-products could be formed *in situ*. In this way the absence of erythema in mice could be explained by the use of the acetone solution instead of a lanolin ointment.

The hypothesis proposed to explain the therapeutic effects of psoralens on psoriasis is based on the idea that the activity is a consequence of the photoreactions with nuclear DNA (Scott *et al.*, 1976), and interference with DNA synthesis (Walter & Voorhees, 1973; Bordin *et al.*, 1976). This may hold also for the therapeutic effects of 3-CPs, since mono-functional furocoumarins also are known to block DNA synthesis. As reported earlier (Swanbeck & Thyresson, 1974) several drugs active on psoriasis are able to induce lesions in mitochondrial DNA. Indeed 3-CPs exerts a preferential action on mitochondrial DNA in yeast as compared to 8-MOP (Averbeck *et al.*, 1978; Averbeck & Moustacchi, 1979). In strictly aerobic cells such an effect may have major consequences both on cell cycle parameters and on cellular differentiation, so it is possible that the therapeutic action of 3-CPs is related to the action on mitochondrial DNA, but some other unknown reactions on other cell constituents may be involved.

In conclusion, it may be said that a study on the genetic effects of different mono- and bi-functional furocoumarins in a lower eukaryote (*Saccharomyces cerevisiae*) taken as a model system, led us to select a derivative of psoralen, 3-carbethoxypsoralen, which exhibits high photo-affinity to DNA, but in contrast to 8-MOP, is only able to form mono-additions. The induced mono-additions were shown to be easily repairable, and they provoke less nuclear genetic events, such as nuclear mutations and mitotic crossovers, but are more mutagenic on mitochondrial DNA.

Tests in mice revealed that 3-CPs is non-toxic, does not produce erythema, or tumours in conditions where 8-MOP is able to do so.

A preliminary study on ten psoriatic patients has demonstrated that this non-carcinogenic but photosensitizing agent, 3-CPs, when applied locally, is as efficient in the clearing of psoriatic lesions as local treatments with 8-MOP. Since 8-MOP is known to be more therapeutically active in oral than in local treatments a continuation of the clinical work with 3-CPs by oral administration must be envisaged but after the necessary pharmacokinetic and toxicological studies.

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Transient impairment of peripheral blood lymphocyte function during PUVA therapy

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SUMMARY

Three parameters of immune function—enumeration of circulating T and B lymphocytes and response of lymphocytes to graduated doses of phytohaemagglutinin (PHA)—were examined serially in eleven patients with psoriasis before, during, and after an intensive course of PUVA therapy. A trend was detected for the lymphocyte response to PHA to fall during the first week of treatment and then rise back towards the pre-treatment level. No alteration was found in the percentage or absolute numbers of circulating T and B lymphocytes. This study shows that routine PUVA therapy can induce an alteration in immune function but that this alteration is slight and short-lived.

Oral psoralen photochemotherapy (PUVA) (Parrish *et al.*, 1974) has been shown to be an effective treatment of psoriasis (Melski *et al.*, 1977). Although a suggested advantage of PUVA over other systemic therapies for psoriasis is the apparent limitation of its effect to the skin, this concept may not be entirely true. Up to 50% of incident UV-A in a fair-skinned Caucasian penetrates into the dermis (Everett *et al.*, 1966), which contains blood vessels and cells of non-cutaneous origin and function. Because these cells may take up psoralens, it is possible that circulating and tissue blood cells could be affected by PUVA therapy resulting in an alteration in their function and viability. Support for this possibility comes from recent reports of a beneficial effect of PUVA in mycosis fungoides (Gilchrest *et al.*, 1976; Roenigk, 1977), the abnormal lympho-reticular cells being cleared from the dermis and epidermis by a course of PUVA therapy. Even more direct evidence is provided by the observation that *in vitro* uptake of tritiated thymidine by unstimulated peripheral blood lymphocytes is decreased immediately following *in vivo* exposure to PUVA of patients with psoriasis (Kraemer & Weinstein, 1977). Lymphocytes play a central role in the maintenance of normal immune function and any treatment that produces an impairment of their function or viability could result in a suppression of immune function. As yet, there have not been any reports of clinical evidence of

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immune suppression in patients, but such an effect might be subtle and escape notice until after many years of PUVA exposures. The present study was undertaken to explore the effect of PUVA therapy on lymphocyte function in patients undergoing a course of treatment. Three parameters were examined: enumeration of T cells, enumeration of B cells and the response of mononuclear cells to graduated doses of phytohaemagglutinin (PHA).

MATERIALS AND METHODS

Eleven patients with plaque-type psoriasis were used in the study. Age range for the group was 27–62 years with a mean of 48 years. The average extent of psoriasis was 50% of the body surface and the duration of the disease ranged over 10–47 years with a mean of 24 years. Two patients had previously received systemic cytotoxic agents, but not within the preceding year and four patients had been using topical corticosteroids, but not more than 25 gm/week without occlusion.

PUVA therapy

8-Methoxypsoralen (approximately 0·6 mg/kg body weight) was ingested 2 h before each exposure to UV-A radiation. The initial dose of UV-A (1·25–1·75 J/cm^2) was determined by the patient's sunburn and suntan history. Treatments were given four times each week: two treatments on consecutive days, a rest day and then two further treatments on consecutive days. The dose of UV-A was increased by 0·5 J/cm^2 every third treatment. Once the patient was free of disease, treatment was reduced to one exposure each week and the UV-A dose was held constant. The details of the radiation source have been described previously (Parrish *et al.*, 1974).

Design of study

In vitro studies of lymphocyte function were conducted on each patient prior to the start of PUVA treatment. These studies were repeated twice during the first or second week of therapy and weekly thereafter during the whole clearance phase of treatment. Blood samples were obtained 24–96 h after the preceding treatment and in each patient this interval was varied over the whole course of examinations. When the patient was free of disease, two final studies were conducted after a month of weekly maintenance treatments. The blood for these last two studies was taken 24 and 168 h after the preceding PUVA exposure. All blood samples were drawn between 8.00 a.m. and 10.00 a.m.

In vitro studies

Thirty to forty millilitres of venous blood were collected in a heparinized syringe. After white blood cell and differential counts were performed, the mononuclear cells were separated on a Ficoll-Hypaque gradient according to the method of Boyum (1968). These cells were washed three times in Hank's buffered salt solution and then divided for the following tests:

(a) *Enumeration of T cells*. The E-rosette test was used as a marker for T lymphocytes. Mononuclear cells were incubated with latex particles (Difco) for the identification of monocytes. After washing the cell suspension, an aliquot was mixed with sheep red cells (Becton Dickinson, Maryland) plus 10% fetal calf serum (F.C.S.) (Gibco, New York) to give a red cell to lymphocyte ratio of 80:1. The cell suspension was incubated at 37°C for 15 min, centrifuged at 4°C for 5 min at 200 **g** and then incubated at 4°C overnight. Cells were resuspended by gentle tapping of the tube and the number of rosette-forming lymphocytes (lymphocytes with three or more adherent red cells) counted in a haemocytometer. Cell viability was evaluated by trypan blue dye exclusion; one hundred viable cells were counted, and the number of E rosettes was expressed as a percentage of viable lymphocytes. The study was performed in triplicate and the results averaged.

(b) *Enumeration of B cells.* The detection of surface membrane immunoglobulins (SmIg) was used as a marker for B lymphocytes. After incubation of mononuclear cells with latex particles, an aliquot of cells was added to a mixture of rabbit anti-human-kappa and anti-human-lambda light chain antisera conjugated with fluorescein (Behring Diagnostics, New Jersey) and incubated in an ice bath for 30 min. After washing three times, the cells were suspended in glycerin and placed on a slide. Lymphocytes were examined by fluorescence microscopy (Zeiss RA/SN with Epi-illumination) for the presence of surface fluorescence.

(c) *PHA stimulation.* Cultures of mononuclear cells were established in microtitre plates and incubated with a range of concentrations of PHA (Burroughs Wellcome, purified HA 17). Each culture contained 2×10^5 cells in R.P.M.I. (Gibco) with 10% F.C.S. and antibiotics (penicillin 200 u/ml and streptomycin 200 μg/ml). Six concentrations of PHA were used, 10, 1·0, 0·5, 0·25, 0·1, and 0·01 μg/cultures, in order to give a range of stimulating doses above and below 0·25 μg which had been found to be the optimal stimulating dose in preliminary experiments. Cultures for each concentration and a control were established in triplicate. After 66 h of incubation at 37°C, 0·5 μCi of tritiated thymidine (New England Nuclear, Boston, Massachusetts) was added and the plates were incubated for a further 6 h. The samples were harvested using an automated harvester (Mash, Microbiological Associates, Washington, D.C.) and the radioactivity of each sample was measured in a liquid scintillation spectrometer (Beckman Model #LS150). The incorporation of tritiated thymidine into DNA was expressed as c.p.m./culture.

Analysis of results

The results were analysed by application of a set of analysis of variance and linear regression models developed for the specific design of this study. Calculations were performed using computer programmes run on an IBM 370/168 computer.

Two types of models were used: (a) univariate models (analysis of variance and linear regression) for analysis of data on T lymphocyte, B lymphocyte, total lymphocyte and total leukocyte counts and the response of lymphocytes to an optimal stimulating dose of PHA; and (b) a multivariate model for analysis of data on the response of lymphocytes to multiple doses of PHA. Two parameters of the response of lymphocytes to stimulation by graded doses of PHA were examined: the shape of the dose–response curve and the magnitude of the response.

Because *in vitro* studies were performed following only about 25% of the PUVA treatments and the schedule for these studies varied among patients, data were grouped by treatment number. For example, a grouping may consist of data from studies performed after treatment number 1–5, 5–10, 10–15, etc. A wide variety of such groupings were examined for each test performed.

RESULTS

Nine of the eleven patients were cleared of psoriasis in a mean of twenty-six treatments (range 18–37) and an average of ten sets of *in vitro* studies were performed on each patient. PUVA therapy was suspended in three patients, in two due to subacute phototoxicity (persistent erythema with scaling and pruritus) and in one patient due to severe nausea and deterioration of his psoriasis while on treatment. One of the patients with phototoxicity was able to resume treatment and was subsequently cleared of skin lesions. Erythema and subacute phototoxicity were the major problems encountered with the regimen of four treatments each week. Apart from the two episodes of subacute phototoxicity, one patient had a persistent mild erythema over a period of twelve treatments and there were nine individual episodes of symptomatic erythema in five of the eleven patients.

The shape of the dose–response curve of lymphocytes to stimulation by multiple doses of PHA

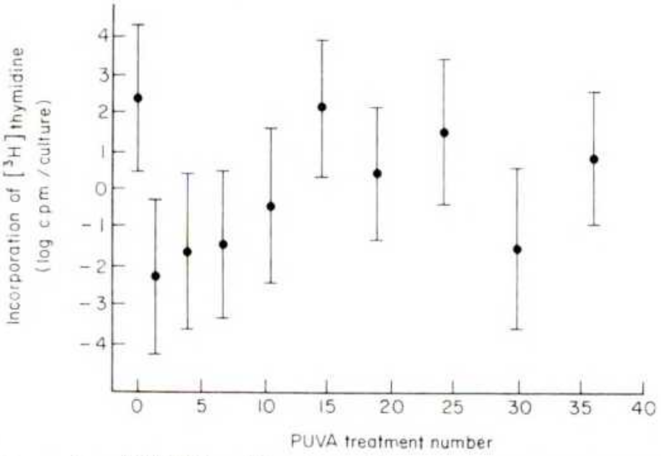

FIGURE 1. Incorporation of [^{3}H] thymidine (± 1 s.d.) by lymphocytes stimulated by PHA (0·25 μg/culture), expressed as difference of log c.p.m./culture from zero (zero = mean of all results from all patients), as a function of number of PUVA treatments. The trend toward decreased incorporation of ^{3}H thymidine observed after the first few treatments was not significant.

was not significantly altered in any of the various groupings of results examined. However, the peak of the curve did change during the course of PUVA therapy and this was evident in both the multivariate and the univariate models used for analysis, i.e. for both multiple and optimal stimulating doses of PHA. The nature of this alteration can be seen in Fig. 1, in which the response to an optimal stimulating dose of PHA is plotted against a grid of ten groupings of treatments. There was a trend for the response to PHA to decrease after the first few PUVA treatments, to return to the baseline level as the therapy continued and to show a decrease in the early phase of maintenance therapy. The decreased response observed after a few treatments reached significance at the 4% level when three groupings of the data were examined (results obtained pre-treatment, after 1–4 treatments and all later results) and these results are shown in Table I. The alteration in the maintenance phase did not reach significance in any grouping examined. Since blood samples for the *in vitro* studies were drawn at varying time intervals after the previous PUVA exposure, the effect of this variable on the results was also examined, results obtained with blood drawn 24 h after an exposure being compared to results obtained with blood drawn after longer intervals. No difference in the results was detected; the trend for an early decrease in the response was still evident and had the same level of significance.

TABLE 1. Incorporation of tritiated thymidine by lymphocytes stimulated by PHA (0·25 μg/culture) expressed as the difference of log c.p.m./culture from zero (zero = mean of all results from all patients). There was a significant difference ($P<0·04$) between the baseline results obtained prior to therapy and results obtained after 1–4 treatments

Treatment number	No. of observations	Incorporation of [^{3}H] thymidine (log of c.p.m./culture)	Standard error
0	11	+0·23	±0·18
1–4	15	−0·36	±0·15
>4	86	+0·03	±0·03

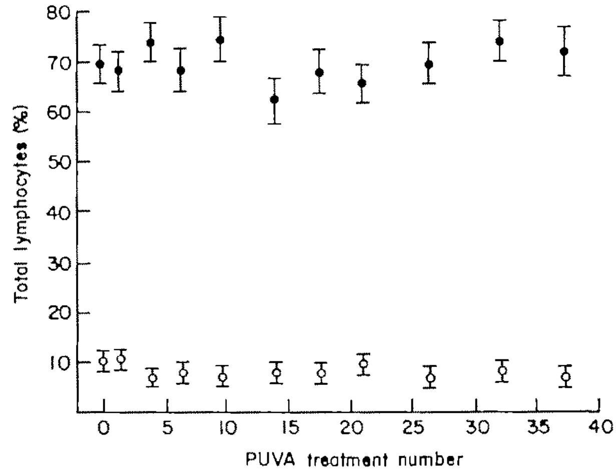

FIGURE 2. Distribution of T (●) and B (○) lymphocytes (± 1 s.d.) as a function of number of PUVA treatments. No significant variation from baseline results was detected in either parameter during the course of therapy.

The course of PUVA therapy did not have any significant effect on the percentage or absolute numbers of T lymphocytes, B lymphocytes, or total lymphocytes, or on the total leukocyte counts in the peripheral blood as determined by application of both types of analysis to the results. Several groupings of data by treatment number were examined and representative results for the distribution of T and B lymphocytes for a grid of eleven treatment groups are illustrated in Fig. 2.

DISCUSSION

This study shows that a course of PUVA therapy for psoriasis does not produce any marked or sustained depression of circulating T and B lymphocytes or of the response of lymphocytes to PHA stimulation. The response to PHA stimulation was the only parameter that showed any alteration which could be attributed to the therapy. There was a trend towards a decreased response to PHA within the first week of treatment and for this response to return towards the pretreatment level while treatment was continued. This trend reached a level of statistical significance only when the results obtained with all doses of PHA in all patients were considered together. The results were also analysed to determine what effect, if any, would have been detected if only a conventional optimal dose PHA stimulation test had been performed, and again there was a similar trend that reached the same level of statistical significance. PUVA therapy alters the magnitude of the response of lymphocytes to PHA, but does not alter the shape of the dose–response curve obtained with graded amounts of PHA.

The effect of PUVA therapy on the response of lymphocytes to stimulation by mitogens has been examined in several studies. The results obtained in these studies have varied. The response of lymphocytes to stimulation by PHA was found not to alter during the course of PUVA therapy in three studies (Ortonne *et al.*, 1978; Wassilew, 1978; Fraki, Eskola & Hopsu-Havu, 1979). Blood samples were obtained either immediately after or immediately before exposures to PUVA; in the latter case it is not possible to be certain of the time lapse after the last exposure to PUVA. The methodology varied with the use of separated lymphocytes in one study (Wassilew, 1978) and whole blood in another study (Fraki *et al.*, 1979), while no details of the methodology were given in the third study (Ortonne *et al.*, 1978). The response of lymphocytes to another mitogen, concanavalin A, was also found to be unchanged during a course of PUVA therapy for psoriasis (Franki *et al.*, 1979).

In contrast, Lischka *et al.* (1977), utilizing $HgCl_2$ as a mitogen, found that the responses of lymphocytes to this agent were decreased after the first week of a course of PUVA therapy but then rose above baseline levels after 2 weeks of therapy. Blood samples in that study were taken either immediately before or after exposure to PUVA. These findings are difficult to interpret because $HgCl_2$ is rarely employed as a mitogen.

The effect of PUVA therapy on lymphocyte sub-populations has also been examined by several groups with a similar variation in the results. Fraki *et al.* (1979) found that the proportion of circulating E-rosette-forming cells rose in a group of patients with psoriasis during a 12-week course of PUVA therapy. The mean result for the twelve patients was 51·3% before therapy and 62·8% after therapy, as compared to 66·7% in a healthy control group. In contrast, Ortonne *et al.* (1978) reported that the proportion of circulating E-rosette-forming cells fell during varying periods of a course of PUVA therapy from a mean of 56·8% to a mean of 36·8%, in a group of patients with psoriasis and other skin diseases. However, since only thirty-six patients were examined prior to PUVA and forty-five patients were examined during PUVA therapy and individual results were not given in the report, it is not possible to be sure whether patients with psoriasis did show a drop in the proportion of circulating E-rosette-forming cells. Cormane *et al.* (1977) reported in an abstract that circulating T cells decreased in ten patients with psoriasis after four exposures to PUVA but returned to baseline values after eight exposures. It is not clear in that report whether these results refer to absolute numbers or proportions of T cells, the method for identifying T cells is not given and the times of blood collections relative to exposures to PUVA are not stated.

There are a number of important variables which could be responsible for the differences in the results obtained in these studies including the methodology employed for the *in vitro* tests, the time of blood collection relative to the last exposure to PUVA and the exposure dose of PUVA. These variables have not been clearly documented in all studies and their absence makes an interpretation of the results difficult. In the present study the techniques of T- and B-cell enumeration were performed according to accepted guidelines (Special Technical Report, 1974) and the graduated dose technique for PHA stimulation was used since it has been shown that it is a more sensitive indicator of immune depression than the conventional procedure employing a single optimal-stimulating dose of PHA (Faguet, 1975). All blood collections were made 24 h or more after exposure to PUVA. Therefore this study does not exclude the possibility that PUVA therapy produces more significant changes, such as a decrease in the proportion of circulating E-rosette-forming cells, which occur within hours of an exposure and persist less than 24 h. The four times a week treatment schedule was selected for the present study to maximize any effect that might be produced. Clinical studies, comparing each of the regimens used in our phototherapy programme, indicated that this regimen keeps patients closest to an erythemal response. The high incidence of erythema and the two cases of sub-acute phototoxicity observed in our study indicate that close to maximal tolerated doses of PUVA were given.

Even when all the variables are clearly defined it is likely that the available *in vitro* tests of immune function will yield variable results in different laboratories. In addition, tests may not detect subtle defects. The conclusion reached from the present study is therefore guarded and limited to a statement that immune suppression produced by PUVA therapy in the course of its routine use does not appear to be of great magnitude and that long-term effects are not readily detectable by current *in vitro* techniques. However, further study of the effects of PUVA therapy on lymphocytes is necessary because some of these cells are long-lived, have a key role in the maintenance of normal immune function and often enter the target area of this therapy. Furthermore, this and previous studies have examined the effect of PUVA therapy on peripheral blood lymphocytes and it is possible that lymphocytes in the dermis have a different sensitivity to the therapy.

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DNA repair synthesis in human skin exposed to ultraviolet radiation used in PUVA (psoralen and UV-A) therapy for psoriasis

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SUMMARY

The ultraviolet radiation used for PUVA therapy stimulated DNA repair activity in normal human skin and in the uninvolved skin from psoriatic patients. The activity detected by autoradiography increased linearly with exposure time. No stimulation was observed when the UV-B component was removed from the incident radiation by filtration through glass. Therefore UV-B damage to DNA was found responsible for the activity detected following exposure to the unfiltered PUVA light source.

PUVA therapy, used recently with success in the treatment of psoriasis (Melski *et al.*, 1977), requires the exposure of patients to ultraviolet (UV) irradiation in the UV-A range (320–400 nm), 2 h after ingestion of 8-methoxypsoralen (psoralen). The therapeutic basis of the treatment may be the impairment of DNA function in epidermal cells by psoralen–DNA photoadducts (Pathak, Kramer & Fitzpatrick, 1974). Psoralen photoadducts are thought to inhibit DNA replication and cell multiplication in the hyperproliferative psoriatic plaque, as they do in bacteria (Oginsky *et al.*, 1959) and in cultured mammalian cells (Baden *et al.*, 1972).

The high intensity light source used for PUVA therapy emits a small but measurable fraction of its total energy in the UV-B range (280–320 nm). Damage to DNA is known to occur in human skin exposed to UV-B (Epstein, Fukuyama & Epstein, 1969) and wavelengths between 285 and 350 nm produce thymine dimers in the epidermal DNA of irradiated guinea-pigs (Pathak, Kramer & Gungerich, 1972).

Thus, at least two types of DNA lesions, photobinding of psoralen and dimerization of pyrimidines, are probably produced in the uninvolved skin of PUVA treated patients. These DNA lesions are subject to excision repair (Hanawalt *et al.*, 1979). *De novo* synthesis of short segments of DNA during the repair process or unscheduled DNA synthesis (uds) can be detected by autoradiography. We have used this technique to determine the extent of repair-stimulating damage produced by exposure of human skin to PUVA light sources in the absence of psoralen.

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MATERIALS AND METHODS

Fresh human skin specimens obtained from mastectomies at surgery and uninvolved skin of psoriatic volunteers were subjected to graded doses of the full spectrum of UV radiation emitted by two commercial PUVA light units. Duplicate fragments of the surgical specimens were exposed to the radiation passed through glass to remove UV-B. Other samples were exposed to the radiation emitted by conventional sun-lamps or the sun.

Light sources

Two PUVA light units were used: General Electric, equipped with forty-eight high output blacklight lamps, F72 T12/BL/HO and Sylvania 64 light box system (GTE Sylvania lighting products group, Danvers, Mass.). While the total energy output from the units varied somewhat with tube usage, up to 99% is delivered in the UV-A range and a constant percentage (between 0·1 and 4% according to the manufacturers) is below 320 nm in the UV-B range. UV-A and UV-B incident energies were measured respectively with LM 301 (Natl. Biol. Corp.) and IL 500 Research (Intern. Light Inc.) radiometers, with the probe placed in the sample position. The UV-B output was confirmed at approximately 0·1% of the UV-A. For example UV-B output was 1×10^{-5} W/cm^2 when the UV-A output was 9×10^{-3} w/cm^2 for the General Electric unit and UV-B was $0{\cdot}82 \times 10^{-5}$ W/cm^2 when the UV-A reading was 11×10^{-3} for the Sylvania unit. Although the light doses are expressed as joules of UV-A (UV-A incident energy in W/cm^2 × time of exposure in seconds) the exposure to one joule of UV-A from the unfiltered source results in exposure to approximately one millijoule of UV-B. When indicated UV-B was removed by two 0·4 mm window-glass plates which were shown to cut transmission below 320 nm. They also absorbed 50% of the UV-A output and therefore twice the exposure time was needed to obtain one joule of UV-A when those filters were used.

Some specimens were exposed to the ultraviolet light emitted by a battery of 8 FS 40 Westinghouse sun-lamp tubes in a walk-in UV-B treatment cabinet (UV-B output: $1{\cdot}3 \times 10^{-4}$ W/cm^2, 10 cm from tubes) and duplicate specimens to sunlight on a clear summer day (UV-B output: $0{\cdot}5 \times 10^{-5}$ W/cm^2).

Material

Slices of the uppermost 0·4 mm thick layer of human skin were removed from fresh mastectomy specimens by keratotome. They were set on sponge rafts moistened with Eagle MEM medium and placed on the cover of a Petri dish for irradiation. Irradiated and control specimens were then cut into 1 × 2 mm fragments and collected in an MEM medium. Sixty minutes after the onset of irradiation, specimens were incubated for 2 h, at 36°C, in 2·5 ml of Eagle MEM medium containing 10 μCi/ml of thymidine-methyl-^{3}H (Schwartz Biochem. spec. act. 5 Ci/mmol). Each sample received a constant gas flow of 95% O_2 and 5% CO_2. Specimens were then rinsed three times with cold MEM medium and fixed overnight in freshly prepared Carnoy's fluid.

Punch biopsy specimens (1 mm in diameter) of clinically normal skin were obtained from the lower back of psoriatic patients prior to and immediately after their exposure to the PUVA light. Biopsy specimens were bisected, placed in MEM medium and incubated according to the technique described above.

Serial sections of the fixed specimens embedded in paraffin were cut at 6 μm and prepared for autoradiography. The slides were soaked in 4% perchloric acid at 4°C for 20 min to extract the unincorporated ^{3}H-thymidine, rinsed in distilled water, dried in air and dipped in NTB-2 Kodak Nuclear Emulsion at 43°C. After a 21 day exposure in the dark at 4°C, the autoradiograms were developed in Kodak D-19 developer and fixed in Kodak rapid fixer. Histological sections were stained through the emulsion with haematoxylin and eosin. In autoradiograms, nuclei undergoing semiconservative replication of DNA during the S (synthetic) phase of the cycle appear very densely labelled while repair synthesis was detected by few silver grains (light labelling) over nuclei. Labelling indexes (Li) were determined as the mean number of densely labelled nuclei per mm of skin surface. The mean grain count over lightly labelled nuclei of the prickle cell layer of the epidermis was taken as a measure of DNA repair synthesis (uds) during the incubation period. The mean grain count was established by counting grains over nuclei in twelve different square micron fields per specimen. Only sections of nuclei appearing in focus with grains in the emulsion (ten or twelve of our fifteen per field) were counted.

Addition of hydroxyurea (10^{-3} M), a metabolic inhibitor of semi-conservative DNA synthesis, to the medium 30 min prior to and during incubation did not affect the light labelling of the nuclei, while the number of grains over cells in the S phase was very much decreased. Treatment of the slides for 24 h at 37°C with DNase (Worthington, Deoxyribonuclease 1, 2000 u/mg) prior to autoradiography eliminated the labelling of nuclei.

RESULTS

Unscheduled DNA synthesis was observed in all specimens of skin exposed *in vitro* or *in situ* to the full spectrum of radiation emitted by either of the two commercial light units. In specimens irradiated *in vitro* the lowest UV dose (1·5 J) produced a grain count over lightly labelled nuclei that was barely distinguishable from background. The level of uds then increased linearly with dose and reached a plateau at about 9 J (Fig. 1). Grains were well focused over nuclei and easily counted (Fig. 2a and b).

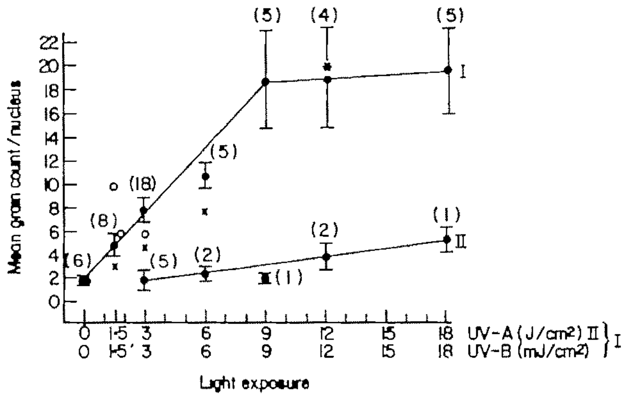

FIGURE 1. Increase in unscheduled DNA synthetic activity as a function of light exposure (●) in mastectomy specimens; (○) in different patients; (×) in the same patient. (I) Exposure to the full spectrum of radiation; (II) to radiation after absorption of UV-B. Numbers of cases are in parentheses. *Synthetic activity in fragment of skin exposed to 12 mJ of UV-B from the conventional fluorescent sun-lamps or the sun.

Irradiation did not alter the morphological aspect of the epithelial cells as observed by light microscopy nor did it decrease the labelling index in the incubated specimens (Table 1). The grain density over cells in the dermis was lower than that in the epidermis of the same specimens. After exposure to 3–6 J, uds in the dermis was easily detected 0·2 mm below the dermo-epidermal junction, appearing most prominently in the endothelial cells of the blood vessels.

Clinically normal skin from psoriatic patients exposed *in situ* to 1·5 to 6 J showed repair activity that was not detectably different from that of mastectomy skin specimens (Figs 1 and 3).

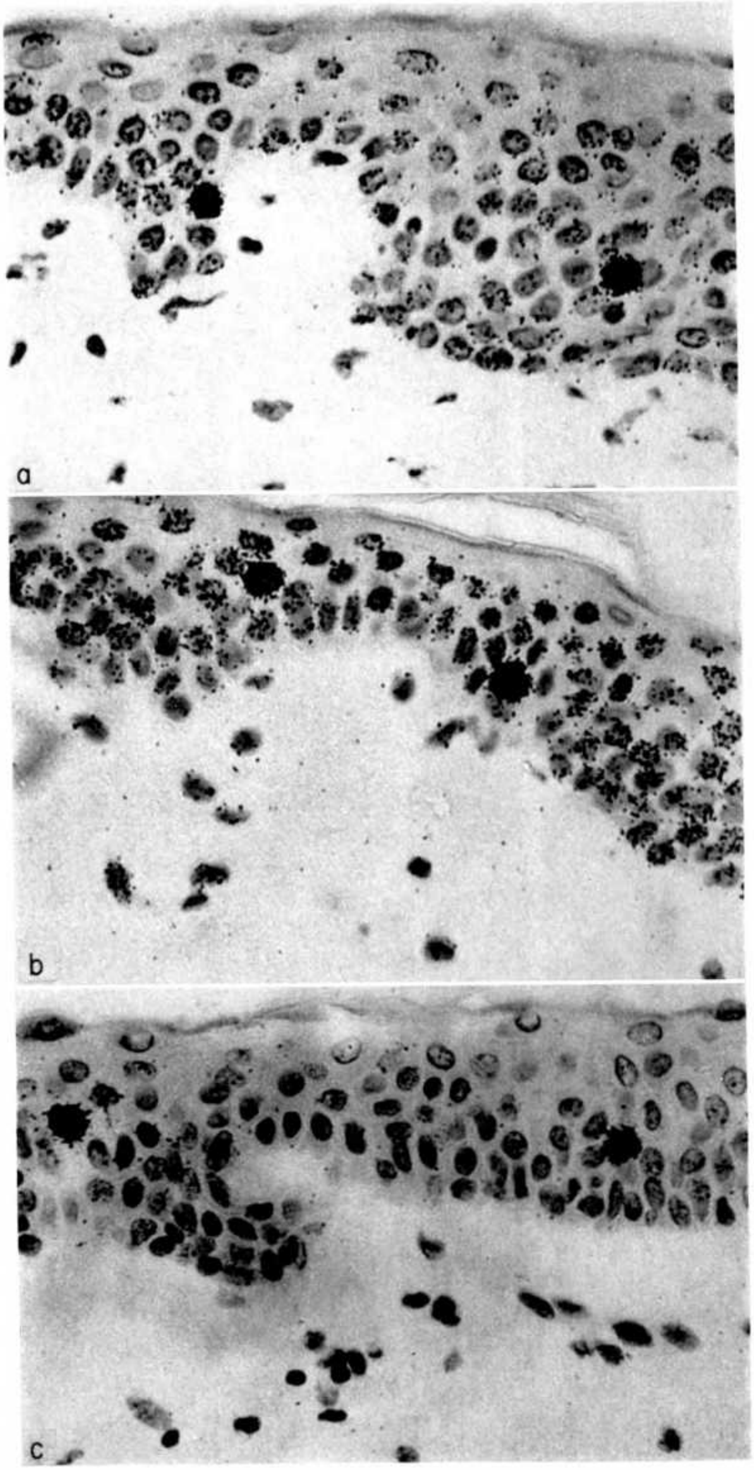

FIGURE 2. Autoradiograms from human skin exposed to (a) 3 J of unfiltered radiation; (b) 12 J of unfiltered radiation; (c) 12 J of UV-A after filtration of radiation through glass (× 300).

TABLE 1. Labelling indexes in mastectomy specimens as a function of light exposure (full spectrum)

(J/cm^2)	0	1·5	3	6	9	12	18
Number of specimens	6	8	14	5	5	3	5
Number of sections	36	39	72	24	34	10	28
Mean Li ± standard error of the mean	5·8 ± 0·9	5·5 ± 2·7	4·8 ± 1·5	4·9 ± 2	5·3 ± 5	5·2 ± 0·3	5·6 ± 2·1

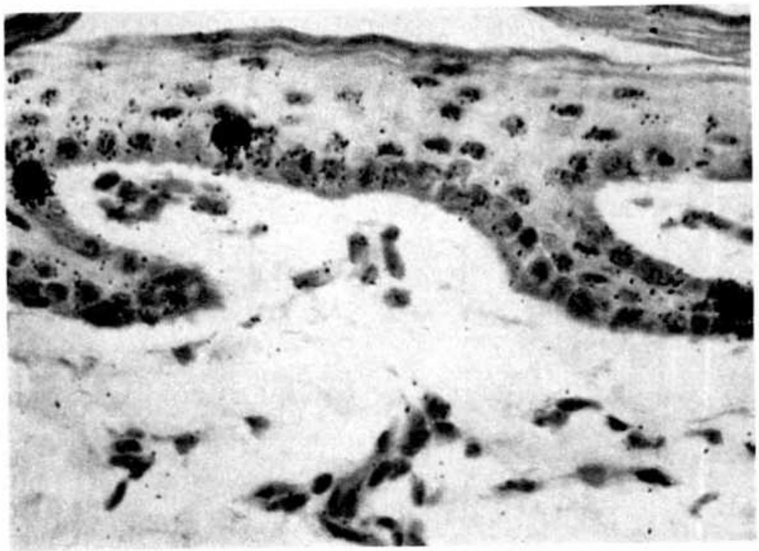

FIGURE 3. Autoradiogram of the uninvolved skin of a patient exposed to 3 J of unfiltered light (× 260).

Duplicate skin fragments from surgical specimens exposed to the filtered light source showed only background levels of repair activity below 12 J of incident radiation (Figs 1–2c). The slight activity observed after higher doses was probably due to an effect of radiation around 320 nm.

In skin fragments exposed to 12 mJ of UV-B from the sun-lamp tubes or the sun, the observed repair activity fell within the range of activity seen after 12 mJ of UV-B from the PUVA units (Fig. 1).

DISCUSSION

The detection of DNA repair synthesis in skin specimens demonstrates that the radiation emitted by PUVA light sources damages DNA in epidermal and dermal cells and it reveals indirectly the depth of penetration of the damaging radiation. The absence of repair activity upon filtration of the radiation through glass indicates that this damage is due to the UV-B component of the source. UV-A does not seem to enhance the stimulation due to UV-B since light sources which contain less UV-A than the PUVA units (e.g. sun-lamps) elicit the same activity per millijoule of UV-B. The repair synthesis stimulation achieved by 20 min of irradiation from the PUVA source is equivalent to that from a 40 min exposure to the sun on a clear summer day.

The detected activity probably represents repair of intrastrand pyrimidine dimers, the principal lesions produced in DNA by UV-B. No effort has yet been made to quantify these dimers in human skin or to compare the kinetics of their release with that of the incorporation of radioactive thymidine into DNA. However, a good correlation has been reported for the rate of repair replication measured by density labelling DNA and the disappearance of thymine dimers in confluent cultures of UV-irradiated human fibroblasts (Smith, 1978).

Repair activity increases linearly with dose and the highest dose tested does not affect the labelling index measured within 3 h of irradiation. This indicates that repair of damage by UV-B alone is very efficient in human skin and that much higher doses are probably required to decrease labelling indices (Epstein *et al.*, 1969). The direct relationship between frequent exposure to UV-B from the sun and skin carcinogenesis is well known (Urbach, 1969). Defects in excision repair mechanisms, as in patients with xeroderma pigmentosum (Cleaver, 1970), are accompanied by an increased sensitivity to the carcinogenic effect of sunlight. Since experiments by Parrish *et al.* (1974) indicate that treatment of psoriatic patients with psoralen and filtered UV-A is clinically effective, it would seem possible to eliminate UV-B from the PUVA therapy. Although human epithelial cells possess an efficient mechanism to repair UV-B damage, a synergism between UV-B, UV-A, psoralen or other DNA-binding drugs used in PUVA and in varied forms of photochemotherapy (Petrozzi & Kligman, 1978; Walter, Stoughton & Dequoy, 1978) might conceivably reduce the repair efficiency. 8-Methoxypsoralen at the physiological dose of 40 μg/ml medium is known to inhibit repair of UV damage in bacteria (Bridges, 1971). The UV-B induced DNA repair activity observed in the uninvolved skin of psoriatic patients completing the clearing phase of PUVA therapy is much below that expected from the dose–response curve established prior to treatment (Bishop, 1977). The possibility that psoralen may protect DNA from UV-B damage or transiently interfere with its repair during PUVA therapy is the subject of continuing investigation.

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Studies on the plasma membrane of normal and psoriatic keratinocytes

1. PREPARATION OF MATERIAL AND MORPHOLOGICAL CHARACTERIZATION

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SUMMARY

A method is described for the preparation of isolated keratinocytes suitable for subsequent biochemical studies. Scanning electron microscopy showed that the maturation process is accompanied by an increase in cell size and a shortening and eventual loss of microvilli. Psoriatic keratinocytes are distinguishable by exhibiting longer microvilli at all levels of maturation.

It is now beyond doubt that the regulation of cell division and differentiation is significantly altered in the psoriatic keratinocyte. Observations at the ultrastructural level (Orfanos *et al.*, 1973; Orfanos, Mahrle & Runne, 1976) have suggested that the fault which is responsible for these abnormalities may be located in the plasma membrane, in particular involving the 'glycocalyx'.

Such a concept is wholly compatible with our knowledge of membrane function in general, for example:

(1) Many of the substances known to control cell cycle parameters are mediated via specific membrane receptors (Emmelot, 1977).

(2) Malignant transformation of cells is accompanied by changes in the cell surface (Nicolson, 1976; Bramwell & Harris, 1978).

(3) Experimental modulation of the cell surface can lead to changes in mitotic rate, cell mobility, cell–substratum and cell–cell interactions (Pardee, 1975; Edelman, 1976; Pastan & Willingham, 1978).

Turning to the molecular level of organization, certain alterations have been reported in the psoriatic membrane which are compatible with a specific defect in glycoconjugate (glycoprotein or glycolipid) metabolism. These include a reduced response of the adenyl cyclase receptor for catecholamines (Yoshikawa *et al.*, 1975; Iizuka *et al.*, 1978), a reduced activity of membrane-bound ATPase activity (Mahrle & Orfanos, 1974) and certain immunological changes (reviewed by Guilhou, Meynadier & Clot, 1978).

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We are therefore studying various aspects of the metabolism of membrane-bound glycoconjugates in the psoriatic lesion. The present paper deals with the preparation and morphological characterization of isolated, viable keratinocytes suitable for biochemical investigation.

MATERIALS AND METHODS

Chemicals

Trypsin (grade III) and ribonuclease (from bovine pancreas, 5 × recrystallized) were obtained from the Sigma Chemical Co., Missouri. TC 199 medium was supplied by Difco (Detroit), penicillin and streptomycin sulphate by Mycofarm, Delft, and the calf serum by the Animal Laboratory of this University. All other chemicals were of Analytic Reagent quality and were supplied by Merck (Darmstadt, Germany).

Skin samples

Control specimens were obtained from healthy, paid volunteers (aged 18–46). Samples of lesion and uninvolved skin were taken from patients with chronic, stable plaques (usually of the back or upper arm) and who had used no local or systemic therapy for at least 2 weeks prior to biopsy.

After cooling the skin surface with an ethyl chloride spray, specimens were cut using a Castroviejo keratotome in conjunction with a steel guard limiting the area of biopsy to about 1 cm^2. Keratotome shims were selected to give an average thickness of 0·2 mm for normal and psoriatic uninvolved skin and 0·3 mm for psoriatic lesion.

Preparation of cell suspension

The biopsy was floated, dermal face downwards, in a plastic Petri dish containing a solution of trypsin (0·25 mg/ml) and dithioerythritol (3 mg/ml) in 10 ml of phosphate-buffered saline (PBS: 137 mM NaCl; 2·68 mM KCl; 8·1 mM $Na_2H\ PO_4$ and 1·47 mM $KH_2\ PO_4$ in distilled, pyrogen-free water). After 30 min at 37°C, the intact specimen was transferred to a second Petri dish containing 2 ml of TC 199 supplemented with 20% calf serum, 200 units/ml penicillin and 100 μg/ml streptomycin sulphate. The dermis was peeled from the epidermis and discarded. The remaining contents of the dish (medium, epidermis and some detached cells) were transferred to a tube and agitated gently ('Vortex' mixer) for 1 min.

After removing the transparent horny layer, 50 μl of a solution of ribonuclease (2·5 mg/ml in phosphate-buffered saline) was added, and the tube incubated for a further 30 min at 37°C. The contents of the tube were finally filtered through a Phywé Korbfilter (pore size 50 μm; Phywé, Göttingen, Germany) and the residue washed with 2 ml medium. The cells were pelleted by centrifugation (100 *g* for 10 min), resuspended in an appropriate volume of medium (usually 0·5 ml) and an aliquot of the suspension counted in a Bürker counting chamber.

All operations were, as far as possible, conducted under sterile conditions.

Preparation of material for scanning E.M.

The cells were washed once with PBS and resuspended at a concentration of 1–4 × 10^6 cells per ml. Fifty microlitres of this suspension was spread on a clean object glass (7 × 7 mm) mounted in a covered Petri dish, and the cells allowed to settle on to the surface of the glass (37°C, 30 min). The attached cells were fixed with 2% glutaraldehyde in PBS (room temperature for 2 h), washed overnight at 4°C, and post-fixed with osmium tetroxide (room temperature for 1 h). After dehydration through graded aqueous acetones, the 100% acetone was replaced by liquid carbon dioxide, the preparation dried using the critical point method and coated with a thin layer of gold. The photographs were made on a Philips 500 Scanning Electron Microscope at an acceleration voltage of 12 kV.

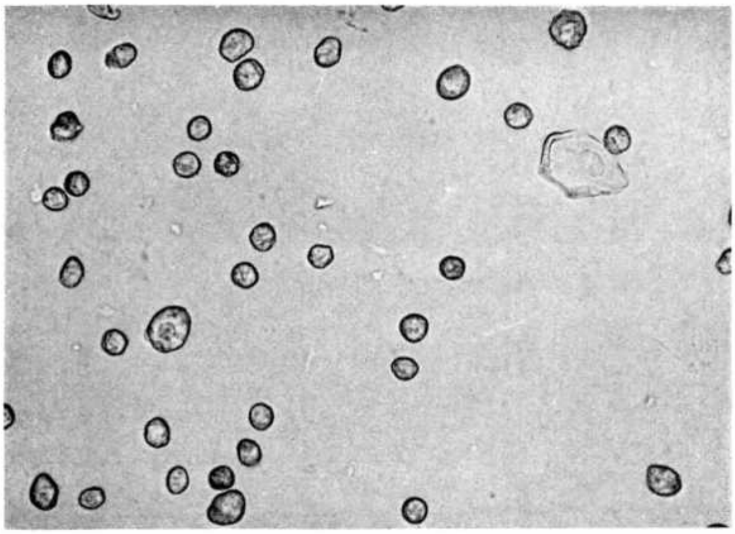

FIGURE 1. A typical preparation of isolated keratinocytes viewed by light microscopy (unstained; × 350).

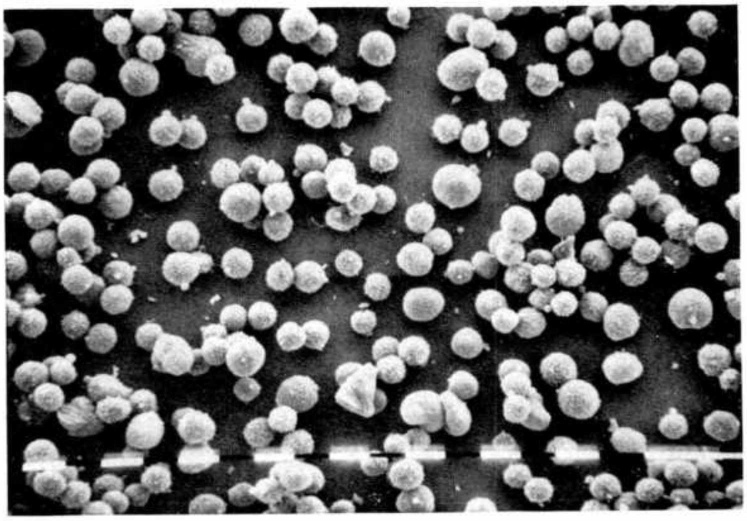

FIGURE 2. A typical preparation viewed by SEM. (Marker bars are each 10 μm.)

RESULTS

In general the preparative method described here yielded $2-4 \times 10^6$ cells. The suspension usually contained a large preponderance of single cells, with occasional (< 10%) clusters of two or more cells (Fig. 1). The viability, assessed by the ability to exclude Trypan blue, was always better than 98%.

Figure 2 illustrates the appearance of a typical preparation from a healthy control as viewed by SEM; note the high degree of morphological heterogeneity. Cells originating from different regions of

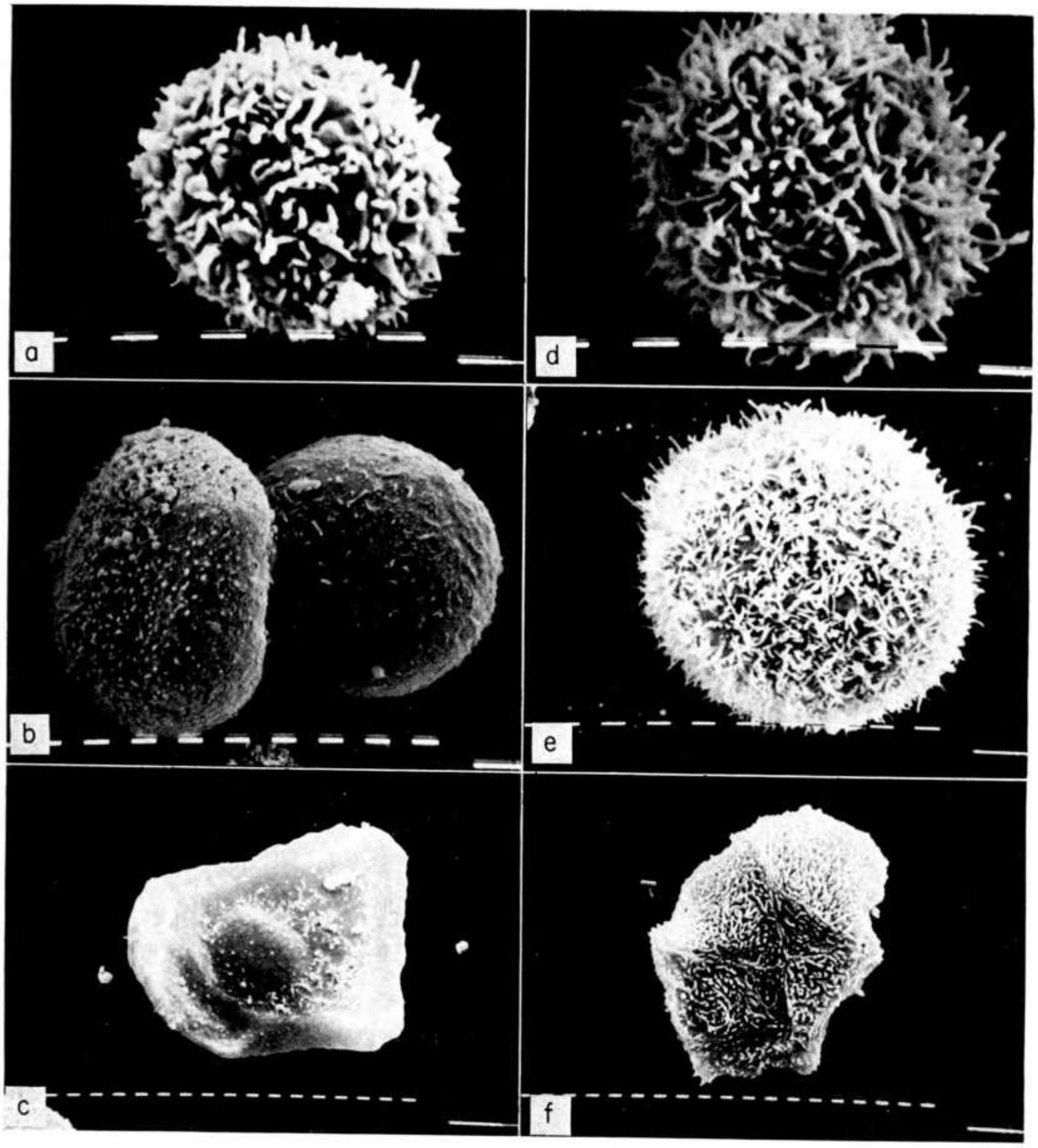

FIGURE 3. Individual cells from normal epidermis (a–c) and psoriatic lesion (d–f). (a) and (d) basal cells; (b) and (e) upper spinous cells; (c) and (f) corneocytes. (Marker bars are each 1 μm; note the differences in magnification between the photographs.)

the epidermis were identified by comparison with similar preparations of trypsinized but intact epidermis.

Figure 3a–c shows the characteristic appearances of basal cells, upper squamous cells, and corneocytes. It is clear that the maturation process is accompanied by a considerable increase in size, a shortening and eventual loss of microvilli, and ultimately by the transition from a spherical to a more or less polygonal shape.

The most clear cut change in keratinocytes from the psoriatic lesion was that cells at all levels exhibited much longer microvilli (Fig. 3d–f). Preparations from the uninvolved skin of psoriatic patients frequently showed similar changes, although to a lesser extent.

DISCUSSION

The use of isolated cells is mandatory for many investigations into membrane structure or function. The practical difficulty when studying solid tissues (especially mechanically tough tissues such as human epidermis) is that any procedure which is sufficiently drastic to separate the cells is liable to cause extensive changes in the plasma membrane. The technique described here has been developed in an attempt to minimize such damage. In particular, the use of dithioerythritol to cleave the desmosomes (Hentzer & Kobayasi, 1976) and the addition of calf serum as trypsin inhibitor before mechanical disruption both represent significant improvements over simple trypsinization. Evidence will be presented in subsequent papers to show that loss of glycoconjugates (in terms of surface carbohydrate or functional receptor groups) does not exceed 20%.

The most significant finding from the SEM studies is that there is a clear and consistent difference between normal and psoriatic keratinocytes at all levels of maturation. Our observations offer strong support for the 'microvillous transformation' described by Orfanos *et al.* (1973) and confirm that our preparative procedure does preserve these abnormalities for further investigation at the molecular level.

ACKNOWLEDGMENTS

The authors wish to thank the many volunteers (with and without psoriasis) who provided us with specimens of skin for use in this investigation.

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British Journal of Dermatology (1979), **101**, 413.

Studies on the plasma membrane of normal and psoriatic keratinocytes

2. CYCLIC AMP AND ITS RESPONSE TO HORMONAL STIMULATION

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SUMMARY

Cyclic AMP levels have been determined for the first time in isolated keratinocytes. Values were more reproducible than those reported using epidermal slices. Evidence is presented to show that damage to hormone receptors is minimal.

Other observations include the following:

(1) Keratinocytes from psoriatic lesions showed reduced 'resting' levels of cyclic AMP as well as a diminished response to adrenaline.

(2) Cyclic AMP levels were maximal in the basal cells, falling dramatically in fully differentiated keratinocytes.

(3) The topical application of a corticosteroid (fluocinolone acetonide) did not modulate the response of adenyl cyclase to hormonal stimulation.

Voorhees & Duell (1971) formulated the concept that certain aberrations of psoriatic epidermis (in particular the alterations of cell cycle parameters and the accumulation of glycogen) might result from a defect in the adenyl cyclase-cyclic AMP cascade. Since this time, ample confirmation has been provided that the ability of adenyl cyclase to respond to catecholamines is indeed reduced in the psoriatic lesion. Nevertheless, the original reports that the cyclic AMP content of the lesion is in fact low have since been denied by several workers, notably Yoshikawa *et al.* (1975a).

All previous investigators have employed either intact epidermis or broken cell preparations, despite the obvious limitations of these materials for receptor studies. The present experiments, using the isolated keratinocyte preparation described previously (Gommans *et al.*, 1979), were undertaken with two main objectives: first, to evaluate this preparation in biochemical terms, and second, to obtain further evidence regarding the role of cyclic AMP in the pathogenesis of psoriasis.

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MATERIALS AND METHODS

Materials

Histamine dihydrochloride and theophylline were purchased from the Sigma Chemical Co. Adrenaline bitartrate was supplied as a sterile solution (1 mg/ml) in physiological saline (O.P.G., Utrecht, NL). Reagents for the measurement of cyclic AMP were obtained from the Radiochemical Centre, Amersham (assay kit TRK 432). All other chemicals were as described previously (Gommans *et al.*, 1979).

The selection of healthy volunteers and psoriatic patients, the method of biopsy and the preparation of isolated keratinocytes have already been reported (Gommans *et al.*, 1979).

General techniques

Approximately 10^6 keratinocytes, suspended in medium (Gommans *et al.*, 1979), were washed twice with phosphate-buffered saline (PBS), resuspended in 200 μl PBS containing 7·5 mmol/l theophylline, and pre-incubated for 20 min at 37°C. The exact cell density of the final suspension was determined.

Following the pre-incubation, the cell suspension was mixed briefly (Vortex mixer) and three samples, each of 50 μl, were withdrawn. One sample was added to a tube, pre-heated to 37°C, containing 20 μl adrenaline (150 μmol/l in PBS); a second was added to a similar tube containing 20 μl histamine (4·5 mmol/l in PBS), and the third sample was injected rapidly into a preheated tube containing 150 μl 0·1 mol/l HCl at 100°C. This last (used to determine the 'resting' level of cyclic AMP) was kept at 100°C for 2 min before cooling to room temperature. The tubes containing adrenaline and histamine were incubated at 37°C for 5 min; aliquots of 50 μl were then injected into 150 μl of 0·1 mol/l HCl at 100°C as before.

All three heat-inactivated samples were sonicated (MSE Ltd, Crawley, Sussex) for 20 s to complete the disruption of the cells, and re-heated for a further 2 min at 100°C. After cooling and centrifuging duplicate 50 μl aliquots of the supernatant were withdrawn from each tube. These were dried *in vacuo* over KOH at 0°C. The dry samples were re-dissolved in 20 μl PBS and (together with appropriate blanks and standards) the cyclic AMP levels were determined using the Gilman procedure (Gilman, 1970). The assay technique described by the Radiochemical Centre was modified by reducing sample and reagent volumes; in our hands the reproducibility was about ±0·05 pmol.

Time curves for hormone stimulation

Preliminary experiments were carried out in which the period of incubation with adrenaline or histamine was varied over the range 0–6 min. In all other respects the technique was as described above.

Receptor regeneration studies

In certain experiments the cells were incubated in the original medium (supplemented with antibiotics and 20% calf serum) at 37°C for periods of up to 12 h after trypsinization. The suspension was then washed twice with PBS and the sensitivity to hormone stimulation determined as before.

Study of individual epidermal layers

Cow snout was used for this investigation because of its much greater thickness (about 0·5 mm) compared with human epithelium. After gross dissection of the skin, successive slices were cut with a keratotome (nominal setting 0·1 mm). Each slice was allocated to one of three pools (superficial, mid-spinous or basal) according to its macro- and microscopical appearance.

The pooled slices were trypsinized and all subsequent procedures carried out as described above.

Pre-treatment with topical corticosteroid

Approximately 80 mg fluocinolone acetonide, 0·2% (Synalar forte, Sarva) was spread over an area of about 2 × 2 cm on one shoulder of healthy volunteers. Occlusive dressing was applied both over the treated area and over a control site on the opposite shoulder. After 24 h the dressings were removed the sites cleaned with alcohol and biopsies taken.

Trypsinization and subsequent procedures were again as previously described.

Protein determination

Aliquots of keratinocytes from healthy controls were washed twice in PBS and disrupted in an all-glass homogenizer (Microwet grinder, Townson and Mercer, Croydon, Surrey). The protein content of the homogenate was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

RESULTS

Time curves for hormonal stimulation

The time courses for the stimulation of normal keratinocytes by adrenaline and histamine are shown in Fig. 1; the results of two independent experiments are illustrated. It is seen that maximum cyclic AMP levels are reached within about 4 min of hormone addition.

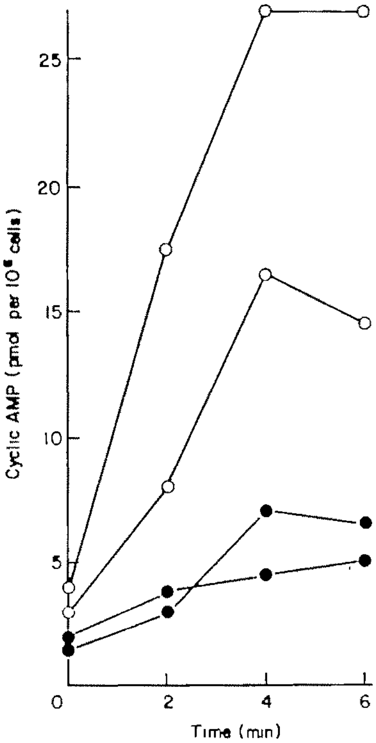

FIGURE 1. Time courses for the stimulation of isolated keratinocytes by adrenaline (○) and histamine (●). Two different preparations were studied in each case.

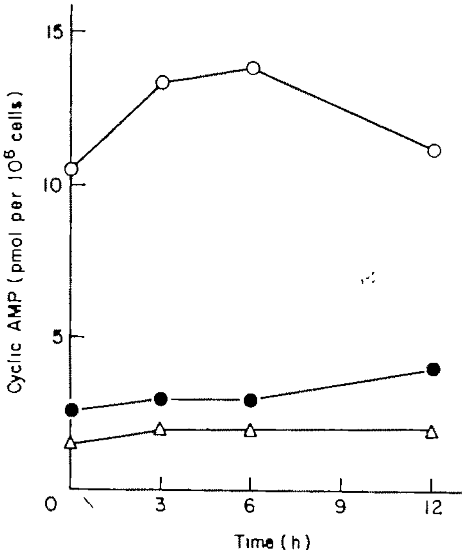

FIGURE 2. Effects of allowing a recovery-period after trypsinization and before determination of hormone sensitivity. (△) resting levels; (○) plus adrenaline; (●) plus histamine.

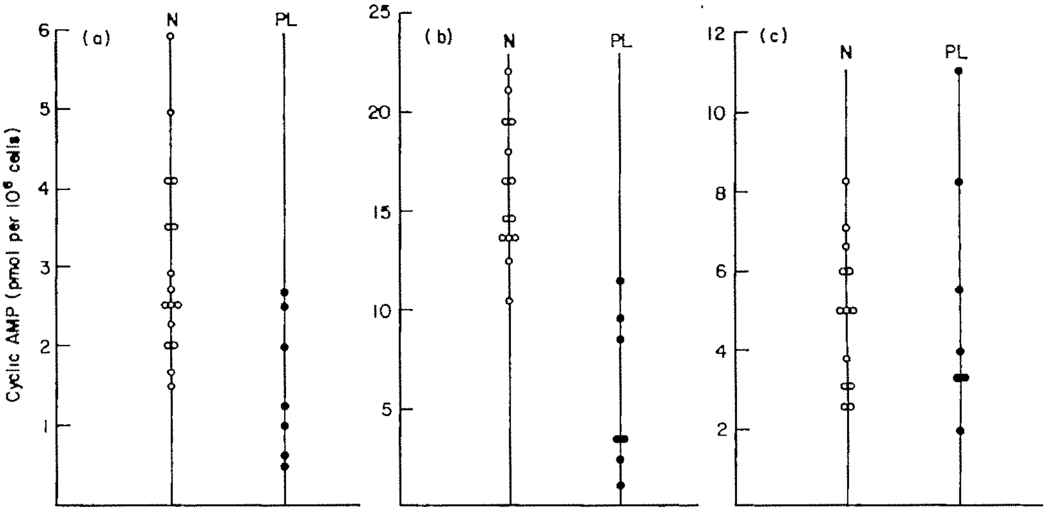

FIGURE 3. Absolute values for cyclic AMP levels in normal (○) and psoriatic (●) keratinocytes. (a) resting levels; (b) following adrenaline stimulation; (c) following histamine stimulation.

Receptor regeneration studies

The effect of allowing the keratinocytes to 'recover' for various periods after trypsinization is illustrated in Fig. 2. It is seen that there is an increase of about 25% in the sensitivity to adrenaline after 3–6 h incubation.

Changes in the psoriatic lesion

Absolute values for cyclic AMP levels ('resting' and following hormonal stimulation) in normal and psoriatic keratinocytes are shown in Fig. 3a–c. Both the resting levels of cyclic AMP and the levels

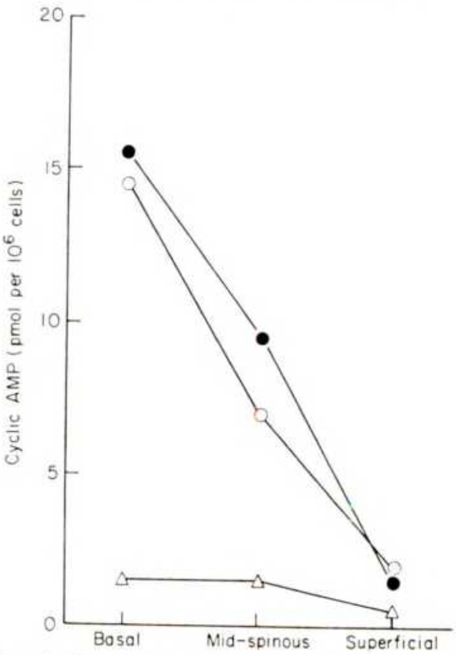

FIGURE 4. Cyclic AMP levels in keratinocytes prepared from different regions of cow snout epidermis. Symbols are as in Fig. 2.

TABLE 1. Effects of pre-treatment with fluocinolone acetonide on 'resting' cyclic AMP levels and sensitivity to hormones

	Cyclic AMP (pmol per 10^6 cells)	
	Treated	Control
Unstimulated	2·8	2·6
Adrenaline stimulated	15·9	17·9
Histamine stimulated	4·7	5·0

following adrenaline stimulation are significantly reduced in cells from the psoriatic lesion (Wilcoxon test, $P<0{\cdot}05$).

Variation with depth

The cyclic AMP levels of basal, mid-spinous and superficial keratinocytes from cow snout are shown in Fig. 4. It is seen that both the 'resting' level and the response to hormonal stimulation are maximal in the basal layer, falling to very low values in the fully differentiated tissue.

Effect of corticosteroid

The values for control and steroid-treated sites are shown in Table 1 (mean of three experiments). The differences are not statistically significant ($P>0{\cdot}05$).

TABLE 2. Hormone response of isolated keratinocytes in comparison to intact epidermis. Note that in this table our results have been recalculated as pmol per mg protein

	Cyclic AMP (pmol per mg protein)	
	Isolated keratinocytes	Intact epidermis (Iizuka *et al.*, 1978)
Adrenaline stimulated	44·1	58·0
Histamine stimulated	12·1	11.7

Protein content

The mean protein content of washed keratinocyte preparations from ten healthy controls was 411 ± 58 μg protein/10^6 cells.

DISCUSSION

The advantages offered by an isolated cell suspension may be illustrated by comparing the present results with those of Iizuka *et al.* (1978), who carried out similar experiments using intact epidermis. The response time of isolated keratinocytes to hormonal stimulation in the presence of theophylline, for example, was less than 4 min compared with 10–20 min for whole epidermis. More important, the wide variations in hormonal responsiveness between individual patients reported by Iizuka *et al.* (1978) were not found in the present investigation. The reason for the improved reproducibility is not clear, but possibilities include the elimination of diffusion limitations, the absence of dermal components, and the removal of endogenous mediators which may be present in the interstitial material.

The most serious reservation regarding the use of isolated keratinocytes is, of course, the possibility of membrane damage resulting from trypsinization. However, the following evidence suggests that such damage is minimal in our preparations:

(a) Our absolute figures for cyclic AMP levels in normal keratinocytes (recalculated to a protein basis, Table 2) are in good agreement with values obtained using whole epidermis.

(b) Experiments in which cells were allowed to 'recover' following trypsinization (Fig. 2) show little increase in hormonal stimulation*.

(c) The reduced response of psoriatic keratinocytes to adrenaline stimulation (about 30% of normal; see Fig. 1b) is consistent with figures obtained using intact epidermis (Voorhees *et al.*, 1974; Yoshikawa *et al.*, 1975b; Iizuka *et al.*, 1978).

Despite considerable dispute, it is now accepted that the cyclic AMP level of psoriatic epidermis *in vivo* is about normal if expressed in terms of DNA, i.e. per cell (Yoshikawa *et al.*, 1975a). Moreover, after pre-incubation with theophylline but in the absence of added hormones the cyclic AMP level of whole epidermis from the lesion seems actually to be elevated (Iizuka *et al.*, 1978). It is therefore of interest that the cyclic AMP content of washed, isolated psoriatic keratinocytes after pre-incubation with theophylline (but again in the absence of added hormones) was lower than normal (Fig. 1a). An hypothesis consistent with these data is that the level of endogenous 'first messengers' in the psoriatic plaque is abnormally high. Alternatively, the difference may be related to oxygen tension; in epidermal slices, the higher oxygen uptake of the lesion will result in hypoxia, and hence higher cyclic AMP levels (Yoshikawa *et al.*, 1975c), whereas in isolated keratinocytes such an effect is unlikely.

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* Evidence will be presented in a subsequent communication to show that turnover of cell surface glycoprotein continues linearly during this period.

The values obtained from individual layers of cow snout epithelium are consistent with histochemical observations that adenyl cyclase activity is maximal in the basal layer (Mahrle & Orfanos, 1975). The data clearly support the concept that cyclic AMP is concerned with the control and co-ordination of proliferation rather than with the events occurring during the process of keratinization. A second point emerging from this work is that cow epithelium resembles pig in showing roughly equal sensitivities to adrenaline and histamine (Iizuka *et al.*, 1976, 1977); human epidermis seems unusual in showing a much lower response to histamine.

The final experiments, in which the responses of keratinocytes to hormonal stimulation were determined following the application of fluocinolone acetonide, were prompted by reports that in some tissues steroids may act by modulating the number of catecholamine and other receptors on the cell membrane (Rajerison *et al.*, 1974; Wolfe, Harden & Molinoff, 1976; Roberts *et al.*, 1977). Such a concept would provide a theoretical basis for the efficacy of corticosteroids in proliferative skin disease. Unfortunately our results (Table 1) do not support the idea of a mechanism of this kind in epidermis, at least for the particular steroid investigated.

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Prolonged BCG treatment of melanoma: does it suppress the immune capacity?

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SUMMARY

The immunological status of seven patients with disseminated melanoma during BCG scarification was followed. As parameters, the total peripheral blood leukocyte and lymphocyte counts, serum immunoglobulin levels, natural ABO blood group antibodies, lymphocyte responses *in vitro* to PHA and PPD, and skin reactivity against PPD and candidin were followed during a period of 2–36 months. The EAC-rosette-forming cells increased and the E-rosette-forming cells decreased during prolonged BCG therapy. The skin reactions and lymphocyte responses showed in most patients conversion from negative to positive or augmentation at the start of the therapy. Later on, however, the values in most patients dropped before disseminated disease became clinically apparent. In the only surviving patient the values first increased, remained high, and after 100 weeks treatment decreased. After 140 weeks' treatment immunological parameters are similar to pre-treatment levels. The possibility that prolonged intensive BCG treatment might eventually suppress the immune system, and thus result in an enhanced risk of dissemination of the disease, is discussed.

After the report by Mathé *et al.* (1969) that non-specific immunotherapy with Bacille Calmette Guérin (BCG) had a favourable effect on patients with acute lymphoblastic leukaemia, this treatment has been under intensive research. Special interest has been focussed on the effects in malignant melanoma. A number of investigators have reported tumour regression after BCG treatment, a decrease in the relapse rate, and improvement of the survival (Bluming *et al.*, 1972; Gutterman *et al.*, 1973a, b; Grant *et al.*, 1974; Eilber *et al.*, 1976). With an increasing number of patients treated with BCG, an increased number of side-effects has also been encountered (Mastrangelo *et al.*, 1976).

It has been established that BCG application either intratumourally or by scarification leads to a rapid increase of immune functions: antigen specific skin reactions become positive, *in vitro* lymphocyte responses against phytohaemagglutinin (PHA) as well as against purified protein derivative (PPD) and other antigens increase, and even elevated serum immunoglobulins have been described (Gutterman *et al.*, 1973a, b; Grant *et al.*, 1974; Mastrangelo *et al.*, 1976; Chess *et al.*, 1973; Lieberman, Wybran & Epstein, 1975; Lui *et al.*, 1975). In most of these studies, the observation period has been

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only a few weeks after the start of the treatment. Gross & Eddie-Quartey (1976) followed melanoma patients for 12 months after the first BCG administration. Their results suggest that immune responses enhanced initially by the BCG become impaired prior to clinical recurrence of malignancy. We have followed some immunological parameters of seven patients with malignant melanoma during BCG treatment for 2–36 months.

MATERIALS AND METHODS

Patients

Six patients with metastatic melanoma of stage II and one patient with a recurrent lesion of the primary site (R.J.) were entered in the follow-up study. The BCG treatment was begun after the patients had been rendered clinically free of the disease by radical surgery as indicated by clinical and roentgenological examination and liver scintigraphies, as well as liver function tests (alkaline phosphatase, glutamyl transferase). In five cases the surgery consisted of radical evacuation of the regional lymph nodes, in one case of radical excision of a local recurrence in the scalp, and in one case of thoracotomy with excision of a pulmonary metastasis. The details regarding the disease at the time of initiation of BCG treatment are presented in Table 1. During the BCG treatment, no chemotherapy was given.

TABLE 1. Clinical details of patients

Patient	Location of the primary tumour	Depth of the infiltration	Location of the recurrent tumour at time of BCG	Further recurrence during BCG therapy	Time lapse on BCG therapy until appearance of new recurrence (months)
H.A.	Upper back	Clark II	Right axillary nodes	Numerous subcutaneous and pulmonary metastases	3
V.P.	Upper arm	Clark II	Right axillary nodes	Subcutaneous, later abdominal metastases	2
R.J.	Scalp	No histology	Scalp	No	
L.P.	Left breast	Clark III	Left axillary nodes	Subcutaneous and cerebral metastases	11
T.V.	Right eye	1/3 of the eye	Left lung (extirpated)	More pulmonary metastases	10
A.H.	Upper back	Clark I	Left axillary nodes	Pulmonary and cerebral metastases	5
K.T.	Left lower arm	No histology	Left axillary nodes (histology: amelanotic melanoma)	Subcutaneous dissemination	11

The follow-up plan

Before the treatment was initiated, and at 1–2 month intervals, the following parameters were assessed for each patient: the total leukocyte count and lymphocyte count, the percentages of E- and EAC-rosette-forming cells, the lymphocyte proliferative responses to PHA and PPD, the serum IgG, IgM and IgA levels and the natural ABO blood group antibody titres. Additionally, the skin tests with PPD and candidin were carried out at monthly intervals. The observation time was 2–140 weeks.

Immunotherapy

The patients were treated with Pasteur high dose special lyophilized BCG, 3×10^8 living organisms per dose. One vial of BCG was administered by scarifications of twenty scratches, each 5 cm long, with a 16-gauge needle on the thigh. The patients were treated weekly for 3 months, every other week for another 3 months, and then monthly until relapse. The scarifications were carried out by one of the authors (I.H.).

The lymphocyte cultures

E-rosette-forming cells were determined according to Jondal, Holm & Wigzell (1972), and EAC-rosette-forming cells according to Blomgren *et al.* (1974) with minor modifications (Nordman, Saarimaa & Toivanen, 1978). The PHA and PPD responses of lymphocytes were determined using the method described by Asantila & Toivanen (1974).

Delayed type hypersensitivity skin tests were carried out and recorded by one of the authors (I.H.). Purified protein derivative (PPD, State Serum Institute, Copenhagen, Denmark), and candidin (Dermatophytin 'O', Hollister-Stier Laboratories, Spokane WA 99220, U.S.A.) were used. For each test, 0·1 ml of solution containing 0·02 μg PPD (Mantoux ITU), equivalent to 1:1000 Old Tuberculin, was applied intradermally. The dose for candidin was 0·1 ml of 1:100 solution. The mean diameter of induration obtained by two right angle measurements at 48 h was recorded in millimetres. Skin tests were repeated at monthly intervals.

RESULTS

The peripheral blood leukocyte counts, the lymphocyte counts, the serum immunoglobulin levels and the natural ABO blood group antibody titres were normal in all patients and remained without any significant changes throughout the follow-up period.

The relative numbers of E- and EAC-rosette-forming cells and their ratio are presented in Table 2. During the follow-up, the number of EAC-rosette-forming cells tended to increase, and after 10 months treatment their number differed significantly from the pretreatment level ($P<0{\cdot}05$ by Student's '*t*'-test). Simultaneously, the E-rosette-forming cells showed a tendency to decrease, although no statistically significant differences from the pre-treatment level were observed. The change was also reflected in the E/EAC-rosette-forming cell ratio, which decreased with prolongation of the therapy from 2·1 to 1·0.

In two cases (H.A. and V.P.) the malignant disease disseminated within 3 months (for details see Table 1). In these patients the immunological functions did not reveal any significant changes. In one of them, both the PPD and PHA responses of lymphocytes, and in the other only the PPD responses showed a slight decline.

The skin reactions to PPD of the five patients surviving for more than 3 months are presented in Fig. 1. The reactions to candidin essentially followed the patterns of those to PPD and are not presented. One patient (R.J.) is still disease-free after more than 140 weeks whereas all others have died of melanoma. In the four cases with recurrent disease, the skin reactions first increased after the beginning of the BCG treatment. One of them (L.P.) showed strong reactions even at the time of relapse. In the other three cases with relapse, the skin reactions had a tendency to decrease before the dissemination. In R.J., with the favourable outcome, the skin tests were originally quite strongly positive, then slightly decreased but remained thereafter at a relatively high level.

The lymphocyte mitogen response to PPD increased in all five patients at the beginning of the BCG treatment (Fig. 2). However, after 5-13 weeks treatment the PPD response diminished, revealing

TABLE 2. Percentages of E- and EAC-rosette-forming cells in the peripheral blood in melanoma patients during BCG scarification therapy. Mean ± s.e.mean are given

Time of sample	E-rosette-forming cells	EAC-rosette-forming cells	Total lymphocyte count	Ratio E/EAC-rosette-forming cells
Before treatment (7)*	64·8 ± 8·4	33·7 ± 4·1	1961 ± 398	2·1 ± 0·4
After 1 month's treatment (7)	59·9 ± 2·9	40·8 ± 4·2	1580 ± 761	1·6 ± 0·2
After 2–3 months treatment (5)	61·8 ± 5·8	34·4 ± 6·9	2020 ± 447	2·2 ± 0·5
After 3–5 months treatment (4)	61·0 ± 6·4	44·5 ± 11·5	2160 ± 448	1·6 ± 0·2
After 5–9 months treatment (4)	74·5 ± 3·1	41·0 ± 2·1	1838 ± 280	1·9 ± 0·1
After 10 months treatment (5)	50·8 ± 7·5	50·0 ± 3·0†	1970 ± 616	1·0 ± 0·2‡
After 15 months treatment (4)	57·5 ± 11·4	59·2 ± 8·0‡	1940 ± 510	1·0 ± 0·3

*Numbers in parentheses refer to the number of patients in the group at the time of analysis.

†$P < 0·02$ }
‡$P < 0·05$ } when compared to the value before treatment, Student's '*t*'-test.

in two patients (R.J. and L.P.) several high peaks later on during the continued scarifications. In all four patients (A.H., T.V., L.P. and K.T.) with relapsing disease the PPD response decreased before dissemination.

The PHA response showed a more confused picture (Fig. 3): in three cases (R.J., K.T. and L.P.) it increased from the originally relatively low values and in two cases (T.V. and A.H.) it decreased at the beginning of therapy from the originally high values. In four cases quite low indices preceded relapsing disease. R.J., the patient still living for more than 32 months without symptoms of melanoma, demonstrated several high peaks in both skin reactions and mitogen indices. The mean survival of the six patients who died from their disease was 7 ± 1·7 months.

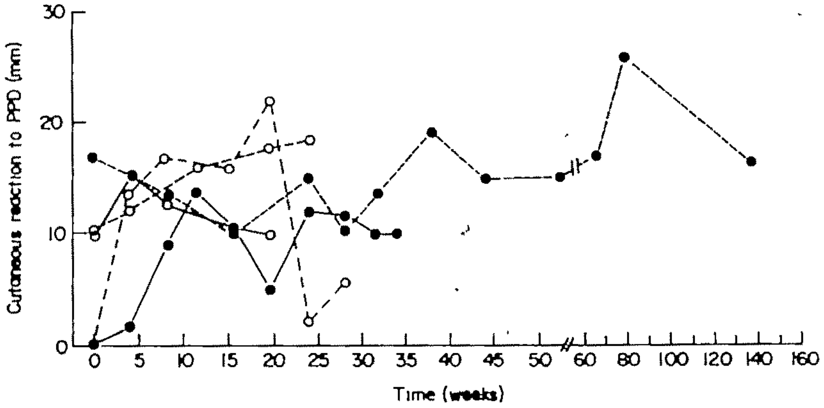

FIGURE 1. Skin reactions of five melanoma patients to PPD during treatment with BCG scarifications. (● - - - - ●) R.J.; (●——●) A.H.(R); (○- - - -○) L.P.(R); (○——○) T.V.(R); (○-—-—○) K.J. (R). R = recurrence of the disease.

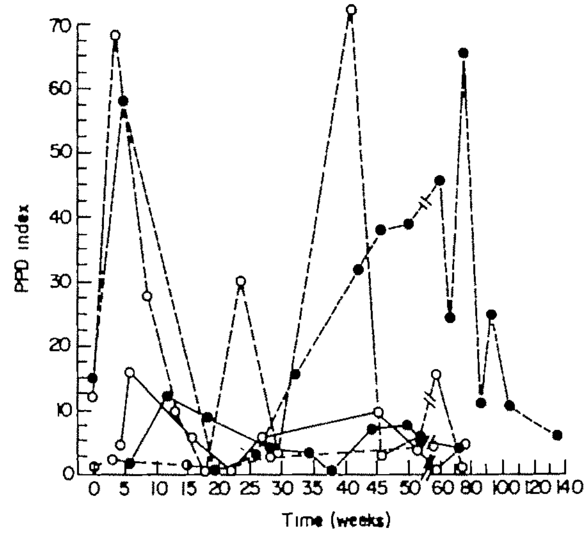

FIGURE 2. The lymphocyte *in vitro* responses to PPD of five melanoma patients during BCG scarification treatment. The results are given as stimulatory indices. (● - - - - ●) R.J.; (●——●) A.H.(R); (○ - - - - ○) L.P.(R); (○——○) T.V.(R); (○-·-·-○) K.J.(R). R = recurrence of the disease.

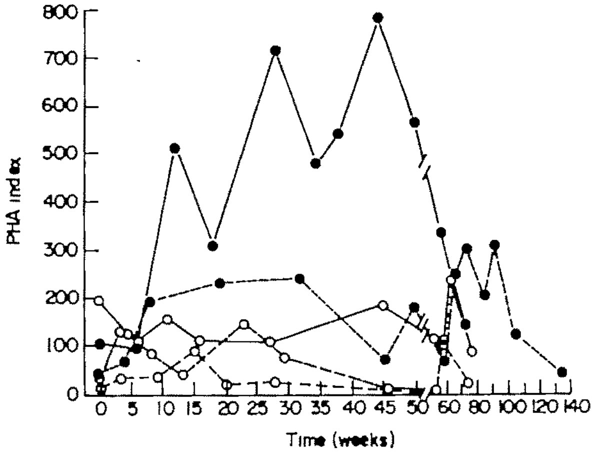

FIGURE 3. The lymphocyte *in vitro* responses to PHA of five melanoma patients during BCG scarification treatment. The results are given as stimulatory indices. (● - - - - ●) R.J.; (●——●) A.H.(R); (○ - - - - ○) L.P.(R); (○——○) T.V.(R); (○-·-·-○) K.J.(R). R = recurrence of the disease.

DISCUSSION

The present study is a long-term follow-up of patients with recurrent melanoma treated with BCG scarifications. The number of leukocytes or lymphocytes did not change significantly during the treatment. We were not able to confirm the observation of Chess *et al.* (1973) that the BCG therapy would increase the serum IgG levels. All the immunoglobulin levels and also the natural ABO blood group antibodies remained essentially unchanged during the whole study.

In order to test the possible effects of the BCG treatment on different lymphocyte populations, the E- and EAC-rosette-forming cells and their ratio were followed. With prolongation of the therapy, the EAC-rosette-forming cells showed an increase from the original 33·7% to 59·2% after 15 months treatment. Since not only B cells are included in the EAC-rosette-forming cell population (Frøland & Natvig, 1973), the significance of the change might be considered questionable. However, simultaneously the number of E-rosette-forming cells and the ratio E/EAC-rosette-forming cells also decreased. We have not observed similar changes in patients with widely disseminated cancer whether or not they received cytostatic therapy (Nordman *et al.*, 1978). Therefore, the changes have to be attributed to the BCG treatment. At present it is not possible to determine exactly what lymphoid cell subpopulations are mostly affected.

The cutaneous reactions to PPD and candidin, and lymphocyte responses to PHA and PPD, showed a clear tendency to rise in the patients who lived for more than 3 months without signs of the disease. The observation is in good concert with a number of studies indicating the immuno-stimulatory effects of BCG treatment (Gutterman *et al.* 1973a, b; Grant *et al.*, 1974; Mastrangelo *et al.*, 1976; Chess *et al.*, 1973; Lieberman *et al.*, 1975; Lui *et al.*, 1975), especially in cases with a favourable outcome (Gross & Eddie-Quatrey, 1976). In most of these studies, however, the effects of the treatment on the patients' immune status have been followed for only a short period of time. With prolongation of the treatment we observed in three out of four patients a decrease in the skin reactivity to PPD and candidin before dissemination of the disease. In all four patients the lymphocyte *in vitro* responses to PHA and PPD showed a decrease preceding the dissemination. The finding is analogous to the observation of Gross & Eddie-Quartey (1976) that the lymphocyte responses to PHA became impaired before clinical recurrence of malignancy in BCG treated melanoma and lung cancer patients. Also, Morton *et al.* (1976) have demonstrated that repeated administration of BCG both in man and in animals may depress cellular immunity as measured by cutaneous delayed reactions to common skin test antigens. In experimental animals, BCG administration has been found to result in an initial increase of the responses of splenic T cells to mitogens, and a later intense suppression (Orbach-Arbouys & Poupon, 1978). Taken together, these observations call for attention when prolonged treatment of cancer patients with BCG is planned. An extended application may, after the initial stimulation, actually lead to an exhaustion of the immune apparatus and thus later increase the susceptibility of the patient to the dissemination of the malignancy. Therefore, monitoring of the immune functions of the patient appears advisable and, if suppression is observed, discontinuation of the treatment for a period should be considered. Furthermore, as also suggested earlier (Gross & Eddie-Quartey, 1976), comparative studies on different administration regimens of BCG treatment with reference to their immunological effects are still needed.

The actual value of the BCG treatment considering the survival of the melanoma patients is difficult to evaluate, as six of the seven cases died 2–11 months (mean 7±1·7) after beginning the treatment. As the 5-year survival of Stage II patients is usually 20% according to Olsen (1966) the mean survival of patients in the present investigation seems to be less favourable.

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Atopic disease and dermatitis herpetiformis

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SUMMARY

Patients with dermatitis herpetiformis were found to have an increased incidence of atopic disorders compared to an age and sex matched control group. The increase in incidence was statistically significant for atopic eczema and any atopic disorder. Serum immunoglobulins (including IgE) were estimated in forty-five dermatitis herpetiformis patients and no significant abnormalities were found.

In recent years an increased incidence of the atopic disorders has been reported in several diseases having in common disturbed immunological mechanisms. These include selective IgA deficiency (Schwarz & Buckley, 1971; Colin-Williams, Chiu & Varga, 1971; Kaufman & Hobbs, 1970), alopecia areata (Miller & Winkelman, 1963; Ikeda, 1965; Penders, 1968), ulcerative colitis (Hammer, Ashurst & Naish, 1968; Jewell & Truelove, 1972; Roberts *et al.*, 1978) and adult coeliac disease (Hodgson *et al.*, 1976).

The jejunal mucosa in dermatitis herpetiformis (DH) is the site of gluten induced damage which, qualitatively at least, is identical to that seen in coeliac disease. Moreover, at a genetic level the diseases appear to be closely related, HLA-B8 (Katz *et al.*, 1972; Scott *et al.*, 1976; Falchuk, Rogentine & Strober, 1972; Stokes *et al.*, 1972) and HLA-DW3 (Solheim *et al.*, 1976; Thomsen, 1976; Keuning *et al.*, 1976) antigens being present in the majority of patients with both disorders. In a recent investigation into the immunological associations of DH we found a statistically increased incidence of atopic disease when compared with a control group (Davies, Marks & Nuki, 1978). In this study with DH we have confirmed and examined in more detail the association of DH with atopic disorders in a larger group of patients. In order to determine whether the two disorders also shared any abnormality in circulating immunoglobulins we have also performed serum immunoglobulin estimations (including IgE) and the radioallergosorbent test (RAST).

PATIENTS AND METHODS

Sixty-one patients with DH were studied. They included twelve patients who had been investigated previously (Davies *et al.*, 1978). The criteria for the diagnosis were clinical, histological and the

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presence of IgA in uninvolved skin on immunofluorescence examination. The latter examination was not performed on three patients. There were thirty-three males and twenty-eight females. Their ages ranged from 8·5–68 years (mean 47 years) for males and from 6·5–73 years (mean 43·5 years) for females. None of these patients was on a rigid gluten free diet at the time of the investigation and in those whose disease was controlled by dapsone, this drug was discontinued for at least 5 days. An identical number of age and sex matched patients (taken from an out-patient population attending for removal of benign naevi and seborrhoeic warts) served as controls. Both DH and control patients were carefully questioned for any past or present history of atopic disease in themselves or their first degree relatives. Specifically, evidence for asthma, hay fever and flexural eczema was sought. Clinical examination was carried out on both groups, note being taken of any type of eczema in addition to current evidence of atopy.

Serum immunoglobulin levels were measured in forty-five of the DH patients. Serum IgG, IgM and IgA were estimated nephelometrically using an automated immunoprecipitation method and IgE by the paper radioimmunosorbent technique (PRIST—Pharmacia Ltd). Using the radioallergosorbent test (RAST—Pharmacia Ltd.) specific IgE to three common environmental allergens—Timothy grass, house dust mite and cat epithelium—was determined where the total serum IgE was in excess of 125 iu/ml.

RESULTS

Atopic disorders

Table 1 shows the numbers of DH and control patients giving a history of atopy. It can be seen that in the DH group there are increased numbers of patients with atopic disorders as compared with the control group. In many of the DH patients the association with atopy was on the basis of a past history and atopic features during the course of the study were either minimal or absent. However, one patient had long standing and active asthma which required daily oral corticosteroid therapy and six out of the eight patients with a history of hay fever continued to have seasonal episodes. Five of the thirteen patients with a history of atopic eczema had active disease intermittently or continuously during the period of study. In all cases this comprised relatively mild, predominantly flexural eczema which had been periodically present since its onset. Two of these patients, in addition, had scalp eczema and to a lesser extent, facial eczema. Four of the five patients had a family history of atopic disease and in two cases co-existing hay fever was present. One patient also had contact allergic dermatitis to nickel. The eczema in these patients was clearly distinguishable from their DH on clinical, histological and therapeutic grounds. In addition to thirteen DH patients with associated atopic eczema there were two others having non-atopic forms of eczema.

TABLE 1. Personal history of atopic disorders in DH and control patients

		Asthma	Hay fever	Eczema	Any atopic disorder	Total
DH patients	Number	2	8	13	17	61
	%	3·3	13·8	21·3	27·9	100
Control patients	Number	1	4	4	7	61
	%	1·6	6·6	6·6	11·4	100
Probability (χ^2 test)		n.s.	n.s.	$0{\cdot}05 > P > 0{\cdot}02$	$0{\cdot}05 > P > 0{\cdot}02$	

n.s. = Not significant.

Table 2 shows the numbers of DH and control patients with a history of atopy in their first degree relatives. Larger numbers of patients having asthma, hay fever, eczema and any atopic feature in their first degree relatives were present in the DH group, although, again probably owing to the relatively small numbers involved, there is only statistical significance for eczema.

Serum immunoglobulins

The means, standard deviations and normal ranges (for our laboratory) of the serum immunoglobulins of forty-five of the DH patients are shown in Table 3. Serum IgG was elevated in four patients, the highest level (21·5 g/l) occurring in a DH patient with associated rheumatoid arthritis. A raised IgA was seen in three patients while a further three patients showed depressed levels. The mean serum IgA level in those patients with atopy was 1·92 g/l and in those without atopy 2·58 g/l. The difference is not statistically significant. Five patients had slightly raised IgM values and in four patients they were below the lower limit of normal. The levels of serum IgE showed a wide range of scatter. Nine out of the forty-five patients (20%) had elevated levels (>125 iu/ml) and details of these are shown in Table 4. It can be seen that six of the nine patients with high serum IgE levels were in fact non-atopics and RASTs to the three common environmental allergens were negative or in low titre. The remaining three atopic patients all had recurrent seasonally active hay fever and showed positive RAST values particularly to Timothy grass. The mean serum IgE level for the DH patients with atopy was 157 iu/ml whereas for the non-atopic patients it was 100 iu/ml. The difference is not statistically significant.

DISCUSSION

This study has demonstrated an increased incidence of atopy in patients with DH and their first degree relatives as compared to a control group. This is in agreement with our previous observations

TABLE 2. Numbers of DH and control patients with a history of atopic disorders in first degree relatives

		Asthma	Hay fever	Eczema	Any atopic disorder	Total
DH	Number	13	12	16	25	61
patients	%	21·3	19·6	26·2	41·0	100
Control	Number	6	7	6	13	61
patients	%	9·8	11·5	9·8	21·3	100
Probability (χ^2 test)		n.s.	n.s.	$0·05 > P > 0·02$	$0·05 > P > 0·02$	

n.s. = Not significant.

TABLE 3. Serum immunoglobulins in forty-five DH patients

Immunoglobulin	Mean ± s.d.	Range	Normal range
IgG g/l	11·6 ± 2·5	5·0–21·5	6·0–16·0
IgA g/l	2·1 ± 1·3	0·4–6·8	1·2–4·0
IgM g/l	1·1 ± 0·55	0·16–2·5	0·5–2·50
IgE iu/ml	110 ± 171	<10–781	0–125

TABLE 4. Clinical associations and RAST studies in DH patients having serum IgE levels > 125 iu/ml

						Atopic disease			
		Serum	RAST*			Patient		Family history	
Sex	Age (years)	IgE (iu/ml)	g6	dl	el	Active	Inactive	(first degree relatives)	Other associated diseases (if any)
F	43	264	0	1	1	—	—	—	Raynaud's phenomenon
M	33	187	0	0	0	—	—	—	Rheumatic fever
M	51	220	1	0	0	—	—	Yes	Rheumatoid arthritis
M	62	242	0	0	0	—	—	Yes	Reticulum cell sarcoma of the jejunum (resected)
F	40	550	0	0	0	—	—	—	—
M	55	368	4	2	0	Hay fever	Eczema	Yes	—
M	58	781	0	1	0	—	—	Yes	—
M	24	252	4	2	2	Hay fever	—	—	—
M	46	703	4	3	0	Hay fever Eczema	—	—	—

* Allergens: g6—Timothy grass.
dl—house dust mite.
el—cat epithelium.
Grades: 0—negative.
4—strongly positive.

(Davies *et al.*, 1978). Serum IgE levels have not been reported previously in DH patients and from this investigation it would appear that the majority of patients have normal values. However, nine out of forty-five patients (20%) with DH did have high levels and although in three patients this was probably related to the associated active atopic disease, in the other six there was no evidence of atopy. The explanation for the elevated serum IgE levels in this latter non-atopic DH group is at present unclear, but it is of interest that in pemphigoid (though not pemphigus) high values have been reported in more than half the patients (Arlesman *et al.*, 1974). Although the DH patients with atopy as a group had a higher mean serum IgE level than the non-atopic patients, this did not reach statistical significance. Most of the patients with a history of atopy did, in fact, have normal serum IgE levels, which agrees with the work of Öhman & Johansson (1974).

Our finding of an increased incidence of atopy in DH is of particular interest in view of the report by Hodgson *et al.* (1976) of an association between atopy and coeliac disease. These latter authors suggested that a damaged jejunal mucosa or abnormal mucosal IgA response in coeliac disease might result in access to dietary allergen(s). Subsequent stimulation of local submucosal IgE synthesis (Colin-Williams *et al.*, 1971) could lead on to atopy. Although this explanation in coeliac disease (and by extrapolation in DH) may at least in part be correct, it should be remembered that the various atopic manifestations often precede symptomatic evidence of either coeliac disease or DH. Some form of genetic linkage between DH/coeliac disease and atopy would appear to be a plausible alternative or additional mechanism to explain these observed associations.

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Skin conductance conditioning with dyshidrotic eczema patients

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SUMMARY

Thirty-three patients with dyshidrotic eczema were trained either to decrease or to increase the electrical conductivity of the skin. Skin conductance has been found to be related to epidermal water content as well as emotional variables, both of which have suggested links to eczema. Subjects trained to decrease skin conductance showed clinical improvement more often than the controls who were trained in the opposite direction. They also showed a significant decrease in measured conductance and anxiety. The controls showed increased anxiety and no significant changes in skin conductance level.

Since its description in 1873 by Tilbury Fox (Simons, 1966), the aetiology of dyshidrotic eczema has not been clearly defined. The fact that it seems to occur more commonly in people with an atopic diathesis has been used to argue for an allergic aetiology. There is much clinical evidence also to support an emotional aetiology (English, 1949). Both factors could be involved in the disorder. Evidence of the emotional component is supported by the common findings of associated hyperhidrosis of the palms and/or soles, since this sweating is generally considered to be emotionally controlled.

If an emotional component is a major factor in the aetiology and maintenance of dyshidrotic eczema, the question remains open as to the mediating mechanism between the autonomic nervous system (ANS) and the end organ acted on in the skin, e.g. blood vessels, sweat glands and/or epidermis. In other words, the question of how the information in the nervous system becomes translated into eczema on the hands remains open.

In recent years, training techniques have been developed which have made it possible to alter autonomic activity with voluntary control by the patient. The techniques have been identified as biofeedback training, or operant conditioning of autonomic activity, in which information is given

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to a patient on a realtime basis about the level and direction of change in autonomic functions (Stern & Kaplan, 1967). With appropriate instructions, subjects are able to learn to alter the physiological activity being measured through mechanisms which are not yet clearly identified.

Since emotional factors, often associated with autonomic changes, may play a role in the aetiology and maintenance of dyshidrotic eczema, reduction in autonomic arousal could provide a basis for clinical improvement. Although the pattern of autonomic change with emotion has been frequently described, Benson, Beary & Carol (1974) provided a succinct description of the changes in terms of what they identified as the 'relaxation response'. They described the general pattern of activity accompanying stimulation of the tropotrophic zone in the CNS, mediated by the parasympathetic nervous system in man. Decreased arousal was identified with decreased heart rate, respiratory rate, decreased oxygen consumption, increased skin resistance (decreased conductance) and increased skeletal muscle blood flow among other changes. Skin conductance was chosen as the function of preference to use in the present investigation since it is a reliable indicator of autonomic activity (Darrow & Gullickson, 1970) in addition to its direct connection with the primary areas manifesting eczema.

One common factor relating skin conductance and dyshidrotic eczema is epidermal water content. Darrow & Gullickson (1970) demonstrated that skin conductance is functionally related to epidermal hydration. Eczema is characterized by increased epidermal hydration, i.e. intercellular and/or intracellular oedema (Lever & Shaumberg-Lever, 1975), and therefore should be related to high skin conductivity. The degree of interdependence of skin conductance and eczema was assessed in the present study which involved training eczema patients to control relative skin conductance.

MATERIAL AND METHODS

Subjects were chosen for evaluation who had received neither topical nor systemic corticosteroids for at least 2 weeks prior to the study and had used no antipruritics, antihistamines or tranquillizers for at least 1 week.

A diagnosis of dyshidrotic eczema was based on the following criteria:

(1) A history of palmar vesicles especially on the thenar and hypothenar eminences and sides of fingers.

(2) No evidence of fungi or an id reaction to fungi elsewhere.

(3) A reasonable attempt to rule out contact dermatitis by history and examination or patch testing when necessary.

A history of atopy was not necessary, and palmar hyperhidrosis was neither a constant nor necessary finding.

The severity and extent of the eczema were rated on six-point clinical scales as shown in Table 1. Severity ratings ranged from 'itching and dryness only' to 'oozing erosion'. Involvement ranged from a 'small area' to 'both hands and feet' at the two extremes. Improvement was similarly scaled as also shown in Table 1, ranging from 'clear' at one extreme to 'much worse' at the other.

Thirty-three patients having the diagnosis of dyshidrotic eczema participated in this investigation. Nineteen were female, ranging in age from 15 to 57 years (mean 28·7), and fourteen were male, ranging in age from 19 to 61 years (mean 32·5). Subjects were assigned randomly to one of two treatment conditions for the investigation. Group A, which received training for decreased skin conductance, included twenty-two patients, eleven female and eleven male. Group B, which was given training for increased skin conductance, included eight female and three male patients.

All subjects were informed that they would be participating in an investigation concerning the effects of skin conductance training on dyshidrotic eczema. Subjects were tested in the Clinical

TABLE 1. Clinical criteria for evaluating the eczema for the patients in the study

Severity	Area involved	Improvement
Grade 1. Itching and dryness only	Grade 1. Small area	Grade 1. Totally clear
Grade 2. Small vesicles, no redness	Grade 2. Less than 1/4 area of one hand	Grade 2. Substantial improvement
Grade 3. Vesicles and redness	Grade 3. Less than 1/2 area of one hand	Grade 3. Slight improvement
Grade 4. Numerous vesicles	Grade 4. Palm and fingers, one hand	Grade 4. No change
Grade 5. Large confluent vesicles	Grade 5. Palms and fingers, both hands	Grade 5. Slightly worse
Grade 6. Oozing erosions	Grade 6. Both hands and feet	Grade 6. Much worse

Research Electrophysiological Laboratory with measures of palmar skin conductance prior to the introduction of training procedures. Subjects were administered the Spielberger State-Trait Anxiety Inventory (STAI) and the Eysenk Personality Inventory (EPI) to get estimates of anxiety and personality characteristics before and after training.

Skin conductance was recorded in an acoustically and electrically shielded chamber with subjects in a relaxed condition, with eyes closed, under both resting and startle conditions. Room illumination was held constant (1·5 foot candles) at the level of the subject's eyes. A 100 Hz sine wave, at a constant current level of less than 8 μA, was injected across the left palm using Grass gold cup electrodes. A conductive medium of 0·05% NaCl in Unibase cream was applied to the electrode site. Skin was prepared by thorough scrubbing of the areas involved with alcohol. Adhesive pads were applied to the thenar and hypothenar eminences, the pads having an exposed area of 0·5 cm^2. The electrode and conductive medium were applied to the open area in the adhesive pads and were anchored in place using surgical tape. Electrodes were allowed a minimum of 10 min to stabilize prior to recording.

Subjects were recorded for resting skin conductance level for 10 min and then were presented with a startle stimulus consiting of a 100 Hz square wave tone at a level of 80 dB re (0·0002 dyn/cm^2). Following the startle stimulus, the recording continued for another 5 min. Skin conductance was recorded in this manner both before and after training.

After the initial baseline recording, subjects were given one of two types of machines to operate and take home. The type A machine consisted of a Radio Shack kit No. 28–182 which provides a tone which decreased in frequency as a relative function of decreased skin conductance. The type B machine was the same kit wired to produce a decrease in tone frequency as a relative function of increased skin conductance. Subjects were instructed in the application of finger tip electrodes which consisted of silver fibre applied to velcro pads. They were told that their task would be to sit quietly for two 15 min periods each day for the following 2 weeks and try to make the tone on the machine decrease in frequency. Questions concerning the best method of achieving this result were given a standard reply that 'Everyone finds that slightly different techniques work best for them, and we are interested in finding out the method which you develop on your own'. The subjects then spent a minimum of 15 min in the laboratory practising with the unit. They were instructed to use no topical or systemic corticosteroids and no antipruritic or antihistamines. They were allowed to use bland, non-medicated lubricants if their hands became too dry.

After a 2-week period, subjects returned to the laboratory for a repeat of their baseline skin conductance recording. After electrophysiological recordings they were clinically evaluated by Dr Miller who was unaware of the type of feedback training received by each subject. The study was continued for an additional 2 weeks. At this time they returned and were re-evaluated with respect to all measurements.

RESULTS

One of the first results noted in the study was the incidence of drop-out among subjects in the B training group, who were the controls in the study. Two of the first three subjects in this group had severe exacerbations of their eczema, had to be withdrawn from the study, and were given medical treatment in the form of oral corticosteroids. Of the nine remaining subjects in this group, five were worse following training, and four showed some improvement. Four of the subjects in the B group reported an inability to control the training machine.

In contrast, the majority of patients in the A training group improved. Of the twenty-two subjects in the A group, only one subject dropped out; four subjects were worse, one subject was unimproved, and sixteen were improved. A comparison of the clinical effects indicated that a significantly higher percentage of group A were improved ($\chi^2 = 4{\cdot}04$, $P<0{\cdot}05$). Only one subject in group A reported an inability to work with the training machine.

The rating scale data indicated that there were no initial differences between the two groups for either severity or area of involvement (Table 2). The improvement scores, however, showed a significant difference between the groups. The A group showed overall improvement, while the B group became slightly worse ($t = 2{\cdot}52$, $P=0{\cdot}02$).

TABLE 2. Clinical rating for the A and B training groups

	Severity		Area		Improvement	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
Group A	2·64	1·08	2·68	1·14	2·88	1·30
Group B	2·60	1·50	2·50	0·53	4·30	1·95

The relationship of training and improvement to psychometric and physiological parameters were assessed using '*t*'-test comparisons of changes from the start of training to the 4-week evaluation (Table 3).

Psychometrics

Both A and B groups showed significant increases in the extraversion scale of the EPI while showing no change in the neuroticism scale. The STAI showed no changes for either of the groups on the 'trait' anxiety scale, or more permanent aspects of anxiety. The 'state' anxiety scale, which is thought to reflect the current status of anxiety, showed a significant decrease for the A group ($t = 2{\cdot}96$, $P=0{\cdot}01$) and a significant increase for the B group between baselines ($t = 2{\cdot}35$, $P=0{\cdot}05$).

A comparison was made between those subjects who improved clinically in the A group ($N = 16$) and all other subjects in the study ($N = 16$). Ninety-three percent of the improved A subjects showed decreased anxiety, while 70% of the others showed increased anxiety ($\chi^2 = 10{\cdot}58$, $P<0{\cdot}005$).

Skin conductance

Subjects in the A group showed significantly lower skin conductance levels during their second recording than their first ($t = -2{\cdot}94$, $P<0{\cdot}02$), while the B group showed no change. Both groups showed a significant reduction in the skin conductance response to the startle stimulus with the B group having the greatest reduction in response ($t = -4{\cdot}11$, $P<0{\cdot}01$).

TABLE 3. Changes in psychometric and skin conductance scores in the training groups

	Group A				Group B			
	Baseline 1		Change at 4 weeks		Baseline 1		Change at 4 weeks	
Variable	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
State' anxiety	37·92	8·81	−6·94*	10·48	35·54	12·30	+4·83	6·82
'Trait' anxiety	37·04	9·02	−2·00	6·98	38·64	11·65	−1·50	5·86
Extraversion	12·83	3·26	+2·72*	3·73	10·36	4·78	+3·83*	2·23
Neuroticism	8·67	4·47	+0·89	3·67	9·73	5·73	+0·83	4·62
Skin conductance level (log μmho)	0·57	0·33	−0·26†	0·34	0·57	0·18	−0·12	0·27
Skin conductance response (log μmho)	0·10	0·09	−0·06†	0·08	0·11	0·09	−0·04*	0·02

*$P<0{\cdot}01$
†$P<0{\cdot}05$.

DISCUSSION

The relationship of skin conductance level to autonomic nervous system functions, and what is regarded as arousal, has long been investigated (Mundy-Castle & McKiever, 1953). Increased skin conductance has repeatedly been demonstrated with increased arousal in acute experimental conditions in relation to personality variables (Roessler, 1973); however, the relationship of changes produced by biofeedback training to changes in arousal has not yet been demonstrated convincingly.

The present results suggest that decreased anxiety was related to improvement in eczema and/or that training to reduce skin conductance may have affected both anxiety and eczema.

Since both groups were receiving attention during the experiment, the similar changes in both extraversion, or outgoing personality style, and reduced skin conductance response could have reflected the effects of attention, adaptation to the experimental environment, the effects of working with changing skin conductance, or some combination of these variables. An important point, however, is that the participation in the research appeared to have some consistent effects.

The A group showed a significant decrease in skin conductance while the other group showed essentially no change. This result is consistent with reports from the subjects. Most patients in the B group reported little success at 'controlling' their machines, while those in the A group reported less difficulty.

It is difficult to determine the clinical effects of the biofeedback training on the dyshidrotic eczema, since normative data have not been published on this disorder. It is apparent that the data may be taken to suggest either that training for reduced skin conductance aided improvement and/or that training for increased skin conductance prevented normal improvement. The prognosis of eczema is variable, ranging from days to months with no normal pattern. In this study we were measuring improvement in specified gradations and not just total remission.

There are a number of questions arising from this study which warrant further investigation. For example, there was no assurance that all subjects had the same amount of practice. Replication with repeated sessions in the laboratory, larger numbers of patients, and a non-training control group could perhaps provide clearer indications of the relationships suggested by the present study.

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The normal trichogram of pubic hair

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SUMMARY

The parameters of the trichogram of the pubic hair were studied with a previously described technique. Measurements were made on males and on non-pregnant, pregnant and post-partum females.

In males and non-pregnant females density and rate of growth steadily decrease with age. There was a higher percentage of telogens in women. Hair thickness was not modified.

The most important findings are related to pregnancy and post-partum which did not induce any change in the parameters of the trichogram of the pubic area. These findings indicate a behaviour of the pubic hair different from the scalp and axillary hair.

This paper reports the results of an investigation into the characteristics of pubic hair to obtain data comparable to those reported for scalp and axillary hairs (Barman, Pecoraro & Astore, 1964; Pecoraro, Astore & Barman, 1964, 1967, 1971; Pecoraro *et al.*, 1964; Barman, Astore & Pecoraro, 1965).

MATERIALS AND METHODS

The investigation was carried out on ninety-five apparently normal Caucasian individuals.

Thirty-two males, thirty-three non-pregnant and thirty pregnant and post-partum females were distributed as follows. The females were divided into three age groups: pubescensce, 10–15 years of age, regardless of the onset of menstruation (five cases); sexual maturity, 16–50 years, with complete development of sexual secondary characteristics (twenty cases); post-menopause, 48–71 years of age, after physiological or surgical menopause, regardless of age or duration (eight cases).

The thirty-two men were grouped similarly, although the age periods showed a wider range: pubescence, 11–18 years of age (four cases); sexual maturity, 19–60 years (twenty-three cases) and senescence, 61–68 years (five cases).

Thirty pregnant and post-partum females were grouped taking into account the variations observed in the trichogram of scalp and axillary hairs during pregnancy and post-partum (Pecoraro *et al.*, 1967,

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1971); from the beginning to the 13th week of pregnancy (seven cases); from the 14–26th week (nine cases); and from the 27th week to delivery (seven cases). The post-partum group was studied from delivery to the 7th week after (seven cases).

The pubic hairs were studied in an area centered at the crossing of two imaginary lines: one sagittal, passing upward from the clitoris or penis; the other, horizontal, through the middle of both inguinal ligaments.

The trichogram parameters were determined as follows:

(1) To establish the rate of growth an area was shaved with a razor. The length of at least five hair shafts, projecting above the skin surface, was measured 5–10 days later. The average of each set of measurements on each individual was then expressed as mm of growth in 10 days.

(2) The state of the hair cycle was determined by plucking the hairs individually, at random, with small forceps. The proximal ends were mounted on a slide with Canada Balsam and observed microscopically. Because it is a short transitory stage the catagen phase was included in the telogen group. About twenty hairs were plucked from each individual and the results expressed as percent of telogens.

(3) Thickness of hairs and density were measured with a microscope specially adapted to the skin surface. Two oculars were used, one of which contained a micrometer scale permitting the measurement of a diameter of at least 0·025 mm. The hairs were grouped into three thicknesses: thick, medium and thin (about 0·1, 0·05 and 0·025 mm respectively).

(4) Density was determined with the other ocular which contained a reticulum that normally allows measurements of density on an area of 0·04 cm^2 (Barman *et al.*, 1964). The lower density in the pubic area, however, required that the study of this parameter be performed with a × 2 eyepiece, which limited the area of study to 1 cm^2 (Pecoraro *et al.*, 1971). Three determinations were carried out in each individual and the results were averaged.

Standard statistical methods were employed for evaluation of the results; correlations were fitted by the least square method (Snedecor, 1956).

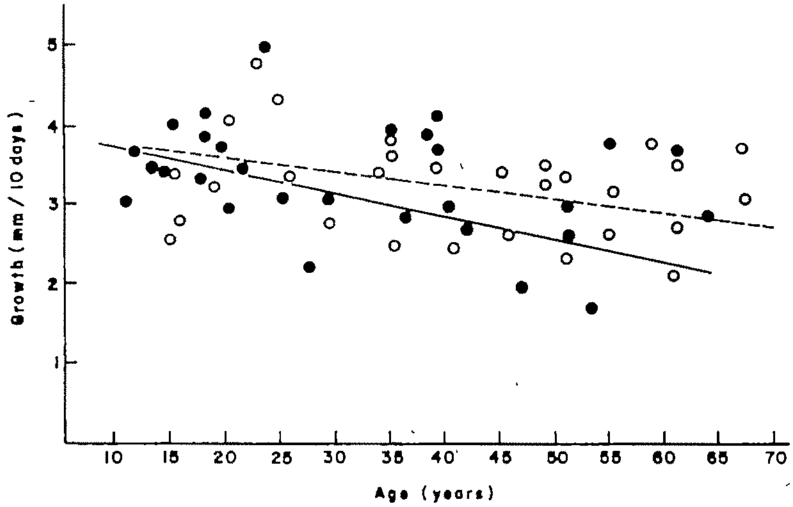

FIGURE 1. Relationship between age and growth rate in males and non-pregnant females. (●, solid line) females; (○, broken line) males.

RESULTS

The results were analysed separately for males and non-pregnant, pregnant and post-partum females.

Males and non-pregnant females (pubescent to senescent and/or menopause)

As shown in Fig. 1 the growth rate is inversely related to the age of the individuals (males: $n = 29$; $P = 0{\cdot}001$; females: $n = 21$; $P = 0{\cdot}05$) without significant differences in slope. The average decay rate is 15 μm/day/year.

Hair density ranged from 7 to 32 hairs/cm^2 in males and from 6 to 31 hairs/cm^2 in females. In both sexes hair density was also inversely related to age (males: $n = 32$; $P = 0{\cdot}001$; females: $n = 29$; $P = 0{\cdot}001$) (Fig. 2).

The difference between sexes is not significant. Hair density decreased at an average rate of 0·31 hairs/cm^2/year.

The state of the hair cycle did not show significant differences between the groups of males and females considered separately. Statistically significant differences were observed when males were compared with females; males sexual maturity vs females sexual maturity: $n = 42$; $t = 3{\cdot}44$; $P = 0{\cdot}01$ and senescence vs menopause: $n = 11$; $t = 2{\cdot}99$; $P = 0{\cdot}05$, the female group showing a higher percentage of telogens.

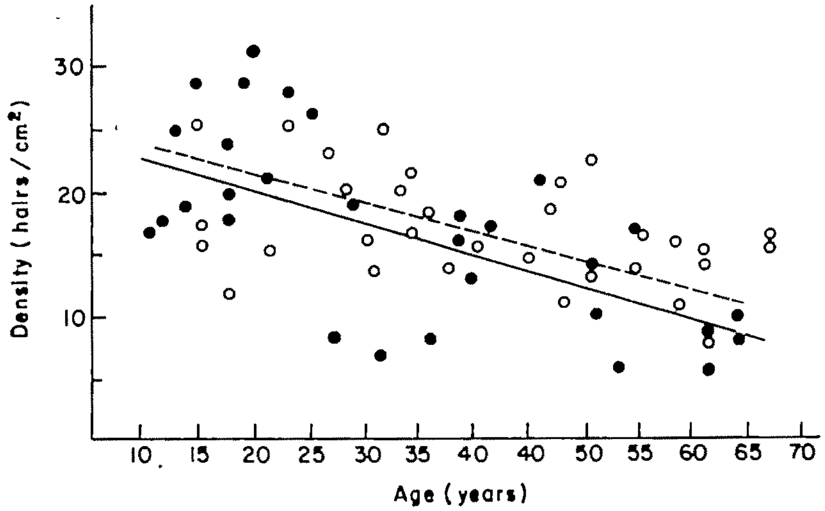

FIGURE 2. Relationship between the age and hair density in males and non-pregnant females. (●, solid line) females; (○, broken line) males.

TABLE 1. Numbers of individuals of different ages with different hair thicknesses

	Males			Females		
	Thick	Medium	Thin	Thick	Medium	Thin
Pubescence	77	23	0	64	30	6
Sexual maturity	65	29	6	69	28	3
Senescence	60	28	12			
Menopause				59	32	9

The study of thicknesses shows a prevalence of thick over medium and thin hairs in both sexes and in all the age groups, as shown in Table 1.

Pregnant and post-partum females

Growth rate, hair density, state of hair cycle and thickness of hairs did not show significant differences either with respect to the duration of pregnancy or in the post-partum period when compared with a group of non-pregnant females of comparable age. No differences were found between those who breast fed their children and those who did not.

DISCUSSION

Pubic hair is one of the six types of hair that shows great uniformity among the majority of individuals of the same racial group (Garn, 1951), allowing comparisons to be made by studying small groups.

Pubic hair seems to be under the influence of the adrenal gland and/or gonads, as inferred by its development at puberty. Later, however, it develops rather similarly to scalp or axillary hair, as shown by the decrease of growth and hair density with age (Barman *et al.*, 1965; Pecoraro *et al.*, 1971).

But pubic hair also shows differences compared to hairs of these other regions. Unlike scalp hair, in the pubic area there are no changes in thickness or state of the hair cycle with ageing. It does not show the spurt of growth that axillary hair shows at sexual maturity. However, the most important differences in the behaviour of pubic hair are noted during pregnancy and post-partum. The increase in the percentage of thick, slowly growing anagen hairs seen in the scalp during pregnancy (Pecoraro *et al.*, 1967) and the decreased rate of growth in the scalp and of hair density in the axilla during the post-partum period (Pecoraro *et al.*, 1971) do not take place in the pubic area, suggesting that the hair of the pubic area is unaffected by these events.

From these data it appears that pubic hair, despite being an important secondary sex character appearing at puberty and before axillary hair (Israel, 1967), is more stable and less sensitive to biological changes in genital activity that normally affect the hair of the scalp and axilla.

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Quantitative microbiology of human vulva

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SUMMARY

The microbial flora of the vulva is described and compared with the flora of the forearm by utilizing the detergent scrub method. Microbial counts were higher on the vulva ($2{\cdot}8 \times 10^6/\text{cm}^2$) than on the forearm ($6{\cdot}4 \times 10^2/\text{cm}^2$). Lipophilic diphtheroids, coagulase negative staphylococci, micrococci, non-lipophilic diphtheroids and lactobacilli formed the dominant flora of the vulva. Streptococci, Gram negative rods and yeasts were also noted. The highest incidence of *S. aureus* was noted on the vulva (67%) followed by perianal (30%), nose (30%), and the forearm (11%).

Although there are numerous reports characterizing the bacterial flora of vagina and perineum, quantitative studies of the normal flora of vulva utilizing the detergent scrub method are absent.

This study investigates the normal flora of vulvar skin and compares it with that of the forearm. Sites known to carry *Staphylococcus aureus* (nose and perianal skin) were compared with vulvar skin for the presence of this organism.

MATERIAL AND METHODS

Eighteen clinically normal women participated in the study. The volunteers were middle-class Caucasian and Filipino technicians and housewives with a mean age of 39 years. The technicians were working in research laboratories not involving direct patient contact. In addition to the vulvar skin (mid-labium majus) and the forearm, the rectum and anterior nares were evaluated for *S. aureus* carriage.

Sampling of organisms

Samples from vulvar skin and forearm were collected by the detergent scrub technique (Williamson & Kligman, 1965). An aluminium cylinder ($4{\cdot}15$ cm^2 in area) was held to the skin, and the surface rubbed with a teflon 'policeman' (a scrubbing device) in 1 ml of buffered Triton X-100 (0·1%) solution for 1 min. Nasal and perianal flora were sampled using calcium alginate swabs. The samples were serially diluted (1:10 to 1:100,000) and subcultured on appropriate media.

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Media

Sheep blood agar, trypticase soy agar (TSA) with Tween 80, eosin methylene blue, crystal violet agar and Sabouraud's glucose agar containing antibiotic (penicillin 20 u/ml and streptomycin 40 μg/ml) were used. Sabouraud agar plates permitted estimation of the total counts of yeasts; TSA with 0·5% Tween 80 was used for lipophilic diphtheroids. Sheep blood agar containing crystal violet allowed estimation of streptococci. Sheep blood agar plates were used to estimate the total count of bacteria. Counts for *Candida albicans* were made on Sabouraud agar and added to the total counts for bacteria. Sabouraud agar plates were incubated at room temperature, others at 37°C for 48 h. Samples from different types of colonies were picked for identification of bacteria. Gram stains were performed on selected colonies. Organisms were classified according to their response to biochemical tests, and by their growth and/or morphology on selective or differential media. Colonial pigment, catalase production, coagulase production, oxidative or fermentative metabolism of glucose, production of spores, and motility were used to identify the organisms.

RESULTS

Percent incidence of microbial flora

These results are shown in Table 1.

TABLE 1. Percent incidence of bacteria at various sites on eighteen subjects

Organism	Vulva (%)	Forearm (%)	*P**
S. aureus	67	11	0·0002
Coagulase negative staphylococci	100	100	1·0000
Micrococci	56	78	0·1030
Alpha haemolytic streptococci	44	6	0·0043
Lipophilic diphtheroids	100	61	0·0043
Non-lipophilic diphtheroids	67	28	0·0146
Lactobacilli	40	10	0·0716
Bacilli	0	17	
Gram negative rods	39	11	0·0560
Yeasts	39	33	0·6655

* P value was performed by using '*t*'-test.

† Ten subjects.

Incidence of *S. aureus* for perianal and nose were 30% for both sites.

The highest incidence of *S. aureus* was noted on the vulva (67%) followed by perineum (30%), nose (30%) and the forearm (11%). The difference between vulvar and nasal carriage is statistically significant ($P < 0·002$). With the exception of micrococci and *Bacillus* all other categories of bacteria were noted higher in incidence on the vulva than on the forearm. *Staphyloccocus saprophyticus* was separated from *Micrococcus* (Baird-Parker, 1974). Methods described by Evans & Kloos (1972) were used to distinguish *Staphylococcus* from *Micrococcus* genus.

Average count of microbial flora (geometric mean)

Counts per square centrimetre were higher on the vulva than on the forearms for all the organisms except *Bacillus* (Table 2). The average total microbial counts/cm^2 were $2·8 \times 10^6$ and $5·4 \times 10^2$

TABLE 2. Bacterial counts for eighteen subjects

Organisms	Vulva (/cm²)	s.d.	Forearm (/cm²)	s.d.
S. aureus	$4\cdot1 \times 10^4$	1×10^5	$1\cdot4 \times 10$	$5\cdot7 \times 10$
Coagulase negative staphylococci	$5\cdot7 \times 10^5$	1×10^6	$1\cdot8 \times 10^2$	$1\cdot9 \times 10^2$
Micrococci	$5\cdot1 \times 10^5$	$1\cdot7 \times 10^5$	$2\cdot9 \times 10^2$	$1\cdot8 \times 10^2$
Streptococci	$3\cdot7 \times 10^2$	$1\cdot4 \times 10^3$	$0\cdot48 \times 10$	$2\cdot3 \times 10$
Lipophilic diphtheroids	$7\cdot9 \times 10^5$	$9\cdot9 \times 10^5$	$1\cdot1 \times 10^2$	$1\cdot5 \times 10^2$
Non-lipophilic diphtheroids	$4\cdot6 \times 10^5$	9×10^5	$1\cdot1 \times 10$	$2\cdot6 \times 10$
Lactobacillus*	$4\cdot6 \times 10^5$	$7\cdot8 \times 10^5$	$0\cdot96 \times 10$	$3\cdot2 \times 10$
Bacillus	0	—	$1\cdot2 \times 10$	$3\cdot2 \times 10$
Gram negative rods	$1\cdot8 \times 10^3$	$5\cdot7 \times 10^3$	$0\cdot12 \times 10$	$0\cdot24 \times 10$
Yeasts	$8\cdot2 \times 10$	$1\cdot7 \times 10^2$	$0\cdot8 \times 10$	$0\cdot9 \times 10$
Total	$2\cdot8 \times 10^6$		$6\cdot4 \times 10^2$	

* Ten subjects.

The *S. aureus* counts/swab for perianal and nose were $3\cdot0 \times 10^3$ and $4\cdot3 \times 10^5$, respectively.

for vulva and forearm, respectively. Lipophilic diphtheroids were the predominant bacteria on the vulva and yeasts were the least numerous. Micrococci, coagulase negative staphylococci and lipophilic diphtheroids were about equally prominent on the forearm. The counts for nasal *S. aureus* ($4\cdot3 \times 10^5$/swab) were higher than the counts for the vulva ($4\cdot1 \times 10^4$/cm²) because the nasal counts were expressed per swab for the nose rather than per cm² as for the vulvar skin.

DISCUSSION

Although the microbial flora of skin is variable, it is relatively consistent in different sites. Anatomical sites with partial occlusion (axilla, perineum and toe webs) yield a greater density of microorganisms than less occluded areas (legs, arms and trunk). Besides quantitative differences, qualitative differences are recognized (Noble & Somerville, 1974; Aly & Maibach, 1977a). The axilla, perineum and toe webs are more frequently colonized by Gram negative bacilli than the drier areas (Noble & Somerville, 1974; Aly & Maibach, 1977a).

The much higher incidence (67%) of *S. aureus* on the vulva was unexpected. The staphylocccal carrier rate for nose and perineum ranges from 10% to 45% in normal population (Noble & Somerville, 1974). Polakoff *et al.* (1967) reported a 13% perineal staphylococcal carrier rate from 361 anaesthetized patients undergoing surgery. A 90% staphylococcal carriage rate occurs in patients with atopic dermatitis (Aly & Maibach, 1977b).

In our results the highest prevalence of *S. aureus* in females is on the vulva and not the nose or the perianal areas. The staphylococcal carriage for nose and perianal skin was comparable to previous reports (Noble & Somerville, 1974). Subjects possessing *S. aureus* on the vulva also yield higher counts of this organism (average counts $4\cdot1 \times 10^4$/cm², range from $2\cdot4 \times 10^2$ to $1\cdot6 \times 10^5$). The data suggest that sites other than nose and perianal skin should be taken into consideration when staphylococcal carriage status is determined. Dispersion of *S. aureus* from vulvar skin may present problems in the field of cross infection; Hill, Howell & Blowers (1974) reported that the perineal region is the main area in dispersal of *S. aureus*, although shedding was much less marked in women than men.

Most categories of bacteria encountered on the vulva were noted in greater density and in higher incidence when compared with the forearm flora. The exception was observed with micrococci and

Bacillus, where the trends were the reverse, i.e. higher incidence on the forearm than on the vulvar skin. However, micrococci when present on the vulva were more numerous than on the forearm. The incidence of alpha haemolytic streptococci (44%) on vulvar skin was striking when compared with the forearm.

Lactobacillus is the predominant flora of the vagina (Corbishley, 1977), and its presence on the adjacent skin was expected. The average total flora of the vulvar skin was much higher ($2{\cdot}8 \times 10^6/\text{cm}^2$) than the total flora of the forearm ($6{\cdot}4 \times 10^2/\text{cm}^2$). This increased density of bacteria on the vulva may be partially water related, as moist skin supports more growth than dry skin (Marples, 1965; Aly *et al.*, 1978). A variable perivaginal flora was seen in a survey of forty-six normal women where soluble alginate swabs were taken from the mucosal surface of the labia and the adjacent thigh (Crowley *et al.*, 1974). These studies were semi-quantitative. The detergent scrub technique utilized in this investigation is superior to soluble swab methods and removes 85% of the bacterial population after a 1 min scrub (Williamson & Kligman, 1965). Further work is needed to determine the pathophysiological significance of the elevated microbial flora, and the factors that prevent or contribute to clinical infection in this anatomical site. Comparable data should also be determined for similar sites in the male.

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Skin diseases in Zambia

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SUMMARY

This is the first report on the incidence of skin diseases in Zambians. A diagnostic analysis of 12,610 patients seen over the past year in the Dermatology Clinic at the University Teaching Hospital in Lusaka, Zambia, is presented. The common diseases as well as the peculiarities are discussed.

The need to develop research centres in various regions of Africa is emphasized in order to provide better insight into the special dermatological problems seen in the black Africans.

Dermatology remains one of the grossly neglected fields of study in the African Continent. There are many countries which so far have not established dermatology as a speciality in their health services. The general physician has to cope as best as he can, but all too often the diagnostic range is limited to a few common dermatoses and steroid cream is the panacea of his armamentarium. The pathologist, whose services are rarely sought, often fails to look beyond acute and chronic dermatitis. Due to this unfortunate situation prevailing in many countries, reports on epidemiology of skin diseases in black Africans have been meagre, apart from the masterly works of reference compiled by Clarke (1959) and Marshall (1964). From these two extensive reports it can be seen that there is a great deal of variation in the incidence of many dermatoses among black Africans in different parts of Africa.

This paper is an attempt to provide a broad outline of the types of skin disorders seen in Zambia.

Zambia is a landlocked country situated in the southern part of Africa with a surface area of about 744,000 km^2. It is roughly the size of France, Switzerland, Austria, Belgium, Luxembourg and Denmark combined. Most of the country is a plateau between 1000 and 1500 m above sea level. The climate is generally dry and hot, the temperature ranging between 12°C and 30°C in different seasons. The total population is about 5·8 million.

There has been considerable intermixing among peoples of different countries in the central and southern parts of Africa, and Marshall (1964) described all these people as one group belonging to the Bantu race. Among Zambians, culturally there is not much to differentiate except that they are further divided by numerous tribes with six officially recognized languages. The traditional tribal culture is slowly giving way to the modern civilization in the fast developing urban areas. However, as far as medicine is concerned the tribal beliefs are hard to change and even today the majority of educated

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Zambians strongly believe in the spiritual basis for disease and do not hesitate to consult the witch doctors. Since skin diseases are rarely life-threatening, people perhaps do not feel the need for strong counter measures performed by the witch doctors. Modern medicine appears to be quite popular for skin diseases in Zambia.

MATERIALS AND METHODS

In 1977, the first dermatology service was established in the University Teaching Hospital in Lusaka. Data relating to skin diseases seen at the clinic over the past year have been collected and analysed. Only black Zambian patients are included in this study.

RESULTS

A total of 12,610 patients, about 7% of all patients attending this hospital, were seen over this period of 1 year. This incidence is similar to that of the Oxford region in England, an area with relatively little heavy industry (Rook & Wilkinson, 1972). Males outnumbered females in the ratio of 1·7:1.

Parasitic disease is the most common skin disorder (Table 1). The majority of cases are of scabies, followed by creeping eruptions in children. Pediculosis, for unknown reasons, is uncommon except for a few cases of pediculosis pubis. Furunculoid lesions of myiasis are common during the rainy season. Pyoderma complicating scabies is the most common presentation seen in children and accounts for the majority of cases seen in our clinic.

TABLE 1. Survey of skin diseases in Zambia in 12,610 patients over the period September 1977 to September 1978. Patients comprised 7870 males (62·4%); 4740 females (37·6%); 8343 adults (66·2%); 4267 children (33·8%)

Diagnosis	Percentage
Parasitosis*	32·2
Pyoderma	20·3
Eczemas	14·7
Mycoses	6·0
Pityriasis versicolor	4·8
Urticaria	3·5
Photodermatoses	2·6
Pityriasis rosea	2·1
Acne vulgaris	2·1
Viral infections	1·6
Malnutrition	1·5
Vitiligo	1·4
Lichen planus	1·4
Alopecia areata	1·1
Keratoderma	1·1
Leprosy	1·1
Psoriasis	1·0
Erythema multiforme	1·0
Secondary syphilis	0·7
Drug reactions	0·7

* Including scabies.

Sweat-induced dermatoses are very frequent. This may be related to the observation that the African skin contains more sweat glands per unit area than the European and also that they sweat more for an equivalent effort (Ladell, 1959). This is further aggravated by the dressing habits of Zambians, which are considerably influenced by the European tradition. Pityriasis versicolor is almost universal in young adults and the relatively small number recorded here represents only those cases with extensive skin involvement and those who are worried about the cosmetic disfigurement. This is similarly the case with athlete's foot and secondary fungal and candida infections. Tinea pedis with pompholyx-like ide reactions on palms and soles is frequently seen. Pseudosycosis barbae due to in-growing of hair and keloidal folliculitis on the nape of the neck are recognized problems in Africans, and Zambians are no exception. Treatment of both these conditions has been a frustrating experience.

Another common condition seen in Zambian women is an acquired dermatosis associated with the use of facial creams containing hydroquinone for its skin lightening effect. A variety of popular preparations are sold over the counter to enthusiastic young women. Their damaging effect after exposure to the sun in producing exogenous ochronosis, pigmented colloid milium and solar elastotic changes has been well documented in South Africa (Findlay, Morrison & Simpson, 1975). These changes are unfortunately irreversible and only an effective official ban can save the ignorant women from the harmful effects of these creams.

Contact allergic dermatitis, so common in the industrial and civilised world, does not seem to be prevalent in Zambia. Cases of industrial dermatitis seem to be meagre and the majority of eczemas seen by us were classified either as one of the constitutional types or unspecified. As already mentioned folklore medicines are not commonly used for skin diseases and untoward reactions to such medicines were noticed in very few cases. One striking case was that of a boy of 15 years who developed a severe primary necrotic reaction to a herbal extract which he was encouraged to apply on his genital area to improve the size of his penis.

Among systemic mycoses chromomycosis is the commonest, followed by cryptotococcosis. Three cases of chromomycosis and two cases of purely cutaneous North American blastomycosis have been seen in our clinic in the past year (Table 2). A few cases of systemic North American blastomycosis have already been reported from this country (Bhagwandeen, 1974).

Although pulmonary tuberculosis is a common disease in Zambia, cutaneous lesions are relatively rare. Only one case of scrofuloderma and two cases of papulo-necrotic tuberculide were seen in this period. Lupus vulgaris has been conspicuously absent. The paucity of cutaneous lesions of tuberculosis in black Africans in general has been mentioned by Marshall (1964). No accurate estimates at a national level are available on the incidence and prevalence of leprosy in Zambia. However, in 1972, 16,005 patients were said to be registered with a prevalence of 4·5 per 1000 estimated population. In our clinic leprosy accounted for 1·1% of all skin cases, a significant prevalence. The incidence of other mycobacterial skin infections cannot be assessed properly due to the lack of adequate laboratory facilities.

Acne, alopecia areata and warts are not as uncommon in our population as in Nigeria (Clarke, 1959). Chronic urticaria is a fairly common and difficult problem since in most instances no causative factor can be identified.

The recurring bullous form of erythema multiforme associated with herpes labialis infection is frequently seen, whereas the less common drug-induced form tends to be severely bullous and merges with the clinical picture of Stevens-Johnson syndrome and toxic epidermal necrolysis. The peculiar localized form of toxic epidermal necrolysis in young Zambians has already been reported (Ratnam, 1978). No definite causative factor has been identified.

The incidence of lichen planus (L.P.) in our cases is about the same as in many other countries in Africa and elsewhere. However, we have noticed two unusual forms which merit consideration.

TABLE 2. Other cases of interest

Diagnosis	Number
Chronic bullous disease in children	10
Localized toxic epidermal necrolysis	9
Stevens-Johnson Syndrome	6
Bullous lesions in lichen planus	4
Pemphigus erythematodes	1
Congenital syphilis	6
Behçet's disease	3
Erythema elevatum diutinum	3
Exfoliative dermatitis	3
Chromomycosis	3
North American blastomycosis (cutaneous)	2
Kaposi's sarcoma	6
Verrucous carcinoma of penis	2
Epithelioid carcinoma	1
Malignant melanoma	1
Systemic lupus erythematosus	1

Firstly, in a few of our cases L.P. presented only with patchy pigmentation and pruritus without the diagnostic papular eruption. Clinically these cases were difficult to differentiate from so-called 'toxic melanosis'. Secondly, we have seen four cases of L.P. with bullous lesions which are said to be rare (Samman, 1972). There has been some confusion as to the nature of these bullous lesions in L.P., particularly with the diagnosis of lichen planus pemphigoides (Sarkany, Caron & Jones, 1964; Stingl & Holubar, 1975).

Pemphigus appears to be very rare since only one case has been observed and there has been no other report of this disease in Zambia.

Acquired porphyria cutanea tarda, the so called 'Bantu porphyria', has been extensively reported in Southern Africa (Gelfand & Mitchell, 1957). In spite of the common occurrence of this condition in Rhodesia and South Africa, it is very rare in Zambia. The case reported here is the first documented in Zambia. Similarly, iron overload syndrome is comparatively infrequent in Zambia. This might explain the decreased incidence of acquired hepatic prophyria, since iron has been implicated in the abnormal porphyrin metabolism (Kushner, Lee & Nacht, 1972; Malkinson & Pearson, 1974). This hypothesis is further strengthened by the fact that traditionally iron vessels are not used by Zambians in making home-brewed alcoholic beverages.

Among cutaneous tumours Kaposi's sarcoma is fairly common. Six cases have been diagnosed with early cutaneous lesions in 1 year. It is important to note that most of the cases in their early stages are referred to the dermatologist. The typical oedema and the small pinkish nodular lesions on extremities form a characteristic clinical picture.

DISCUSSION

The practice of dermatology in Africa provides many difficulties unless one becomes conversant with the inherent peculiarities of the skin of the Africans. The effect of the tropical environment, which causes a large assortment of infections, has also to be considered. Many common skin disorders may present differently and also there are several conditions which appear peculiar to the African. Some of these like ainhum, pseudosycosis barbae, keloidal folliculitis etc. have already been alluded to. We believe there are many more yet to be identified.

The rapid scientific advances achieved during recent years in dermatology appear somewhat less relevant as far as tropical dermatology is concerned. In many countries like Zambia, with inadequate facilities, it is an herculean task to study dermatological problems in depth. Further fundamental research into the racial and the equally important geographical factors is essential to achieve a better understanding of various skin disorders in the African. There is a great need to develop diagnostic research centres in various parts of Africa.

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Male pattern alopecia and coronary artery disease in men

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SUMMARY

The prevalence of male pattern alopecia, coronary artery disease, hypertension and smoking habits were studied in 478 male Caucasian hospital in-patients, over the age of 20 years.

No association was shown between coronary artery disease and either male pattern alopecia, premature male pattern alopecia or male pattern alopecia with a positive family history.

There are conflicting reports on the relation between male pattern alopecia (M.P.A.) and coronary artery disease. Cotton *et al.* (1972) showed that M.P.A. is associated with an increased risk of myocardial infarction but Ben Halim *et al.* (1978) failed to confirm this association.

As M.P.A. occurs commonly in Caucasian men such a positive association with coronary artery disease would be of great importance.

The relation between M.P.A. and clinically overt coronary artery disease was studied in a hospital population.

PATIENTS AND METHODS

Four hundred and seventy-eight Caucasian men, over the age of 20 years, in the medical and surgical wards of the General Infirmary, Leeds were studied. Patients with diabetes mellitus were excluded.

Male pattern alopecia (M.P.A.) was graded using the same criteria as described in the paper by Ben Halim *et al.* (1978). Grade III was taken to represent a significant degree of baldness.

Each patient with M.P.A. Grade III or greater was asked:

(1) Age at which significant hair loss started (within a 5-year period).
(2) Whether two or more other male members of his family had a similar or greater degree of M.P.A. (family history of M.P.A.).

In all patients the following factors were assessed:

(1) Presence of clinically overt coronary artery disease—defined as (a) a documented myocardial infarction, or (b) patients receiving medical treatment for angina pectoris.

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TABLE 1. The prevalence of clinically overt coronary disease and of male pattern alopecia Grade III or greater in each decade

Decade	Total no. in each decade	No. with C.A.D.	No. with M.P.A.	No. with C.A.D. and M.P.A.	Significance (χ^2)	% M.P.A. in C.A.D. group	% M.P.A. in non-C.A.D. group	Significance (s.e. difference %)
20–29 + 30–39	81	3	6	0	n.s.	0	7·7	—
40–49	75	20	20	6	n.s.	30	25·5	n.s.
50–59	122	31	38	15	n.s. ($0{\cdot}05 > P$)	48·3	25·3	n.s. ($0{\cdot}05 > P$)
60–69	112	42	44	21	n.s.	50	32·9	n.s.
70–79 + 80–89	88	31	44	19	n.s.	61·3	43·9	n.s.

C.A.D.—Clinically overt coronary artery disease.
M.P.A.—Male pattern alopecia Grade III or greater.
n.s.—Not significant ($P > 0{\cdot}01$).

(2) Presence of hypertension—defined as patients receiving anti-hypertensive therapy.

(3) A cigarette smoker was defined as having a current regular daily intake of ten cigarettes, or more, during the previous 3 years.

RESULTS

The results were analysed using the chi-squared test with Yates's correction.

Table 1 shows the prevalence of clinically overt coronary artery disease and of M.P.A. Grade III or greater in each decade.

The results show no association between the prevalence of clinically overt coronary artery disease and of M.P.A. Grade III or greater, at any age.

The prevalence of M.P.A. Grade III or greater in patients with and without clinically overt coronary artery disease is also shown in Table 1. There is no significant difference in the prevalence of M.P.A. Grade III or greater between these two groups (analysed using method of standard error of difference between percentages).

TABLE 2. Distribution of patients with hypertension and of smoking habits within the population studied

	Group with M.P.A. Grade III or greater		Non-M.P.A. group	
	Clinically overt coronary artery disease		Clinically overt coronary artery disease	
	Yes	No	Yes	No
% with hypertension	18/61 (29·5%)	20/91 (22%)	25/66 (38%)	49/260 (19%)
% smokers	31/61 (51%)	48/91 (53%)	17/66 (26%)	116/260 (44·6%)

Table 2 shows the distribution of patients with hypertension and of smoking habits within the population studied. There is no significant difference in the prevalence of hypertension between the two groups. Comparing the patients with clinically overt coronary artery disease, there is a significantly higher number of smokers in the group with M.P.A. Grade III or greater ($P<0\cdot01$). The results in Table 2 were analysed using method of standard error of difference between percentages.

The patients with M.P.A. Grade III or greater were divided into two groups depending on whether significant hair loss had occurred before the age of thirty years (premature M.P.A.). Thirty-nine percent of fifty-nine patients with premature M.P.A. had clinically overt coronary artery disease, compared with 45% of the remaining ninety-three. There is no evidence of any association between premature M.P.A. and clinically overt coronary artery disease.

Only sixty-nine patients with M.P.A. Grade III or greater were able to give a definite answer to the question on family history of M.P.A. Of this group, 49% of thirty-seven patients with a positive family history had clinically overt coronary artery disease compared with 53% of thirty-two patients without a family history of M.P.A. There is no evidence of any association between M.P.A. with a positive family history and clinically overt coronary artery disease.

DISCUSSION

M.P.A. is induced by androgenic stimulation of hair follicles predisposed to this response by the interdependent influences of genetic factors and of ageing. Genetic factors also influence the age of onset of baldness and the pattern of its extension (Rook, Wilkinson & Ebling, 1972). Cotton *et al.* (1972) showed a positive association between clinically overt coronary artery disease and M.P.A. Criticism of this work has been made elsewhere (*British Medical Journal*, 1972; Ben Halim *et al.*, 1978). Ben Halim *et al.* (1978) and the present study have failed to confirm this association.

M.P.A. is not a risk factor for the development of coronary artery disease. In Caucasian men both M.P.A. and coronary artery disease occur commonly and there is no clear evidence of any association.

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Bowenoid transformation of seborrhoeic verrucae (keratoses)

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SUMMARY

Seborrhoeic verrucae (keratoses) are considered to be benign lesions. Occasional bowenoid transformation of these benign growths has not been widely recognized. Seven cases of seborrhoeic verrucae with bowenoid transformations are presented to alert the clinician and the pathologist to this occurrence.

Seborrhoeic verruca (keratosis) (S.V.) has been accepted as the term designating a verrucous papillomatous epidermal growth with well-known clinical and histopathological presentations. The prominent dermatology (Caro, 1975; Domonkos, 1971; O'Quinn, 1974; Sanderson, 1972) and dermatopathology texts (Lever & Schaumburg-Lever, 1975; Montgomery, 1967; Gray, 1972; Pinkus & Mehregan, 1976a) consider this growth to be a benign lesion.

On occasions, lesions of S.V. may show degenerative changes resembling Bowen's disease (carcinoma *in situ*). The possibility of this change has not been widely noted. Here seven cases of S.V. with bowenoid transformation are presented to alert the dermatologist and the dermatopathologist to the possibility of this type of dysplastic change.

MATERIAL AND METHODS

Among 3000 seborrhoeic verrucae received in our dermatopathology laboratory for routine processing and diagnosis seven cases of S.V. with bowenoid transformation were diagnosed. The clinical presentations of these seven cases are summarized in Table 1. Routine processing of the tissue specimens was done, and 8 μm sections were stained with haematoxylin-eosin (Pinkus & Mehregan, 1976b) and acid-orcein-Giemsa (Krobock, Rahbari & Mehregan, 1978) stains.

HISTOPATHOLOGY

The periphery of the lesions (Fig. 1) shows typical changes of S.V. Further into the tissue the papil-

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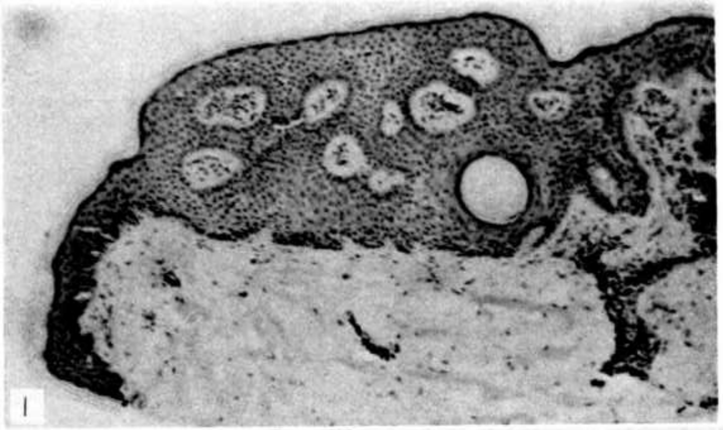
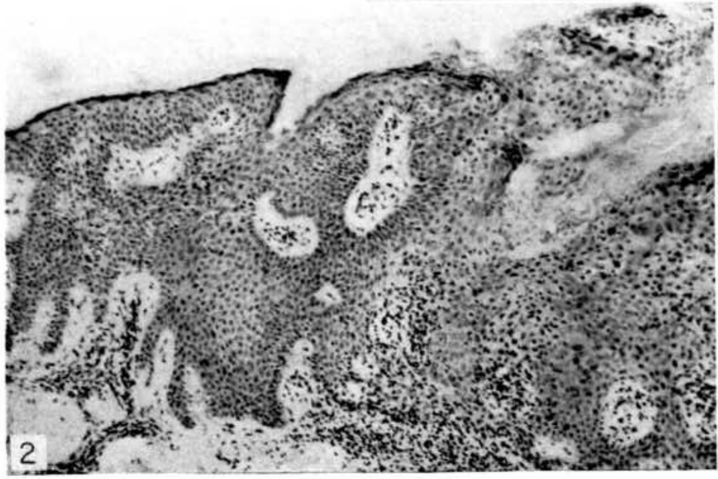




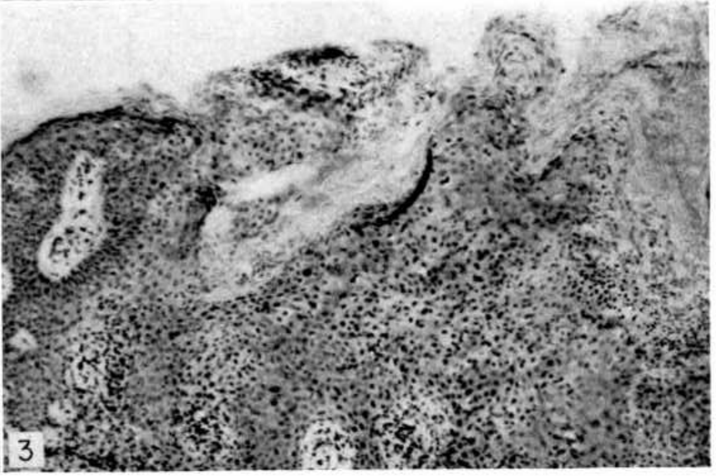


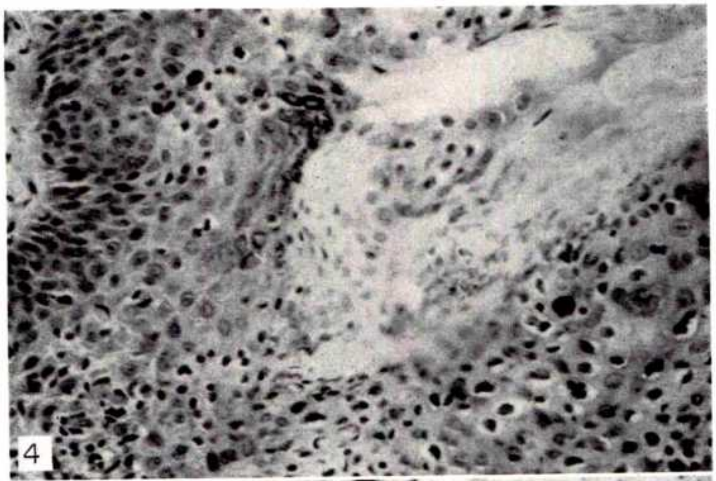
FIGURE 1. Seborrhoeic verruca with typical features (H & E, × 80).

FIGURE 2. Seborrhoeic verruca with early disruptive changes (H & E, × 80).

FIGURE 3. Bowenoid transformation of seborrhoeic verruca (H & E, × 80).

TABLE 1. Clinical details of seven patients with seborrhoeic growths with bowenoid transformation

Cases	Age/sex	Location	Clinical diagnosis
212,066	59/M	Right inner canthus	Seborrhoeic keratosis
289,281	69/M	Right elbow	Seborrhoeic keratosis
298,717	35/F	Right shoulder	Compound naevus
302,149	62/F	Left lateral trunk	Seborrhoeic keratosis
308,755	74/F	Right preauricular	Seborrhoeic verruca *vs* basal cell epithelioma
317, 340-B	61/M	Back	Irritated seborrhoeic keratosis *vs* naevus
333,053	49/F	Nose	Seborrhoeic verruca *vs* basal cell epithelioma *vs* benign naevus



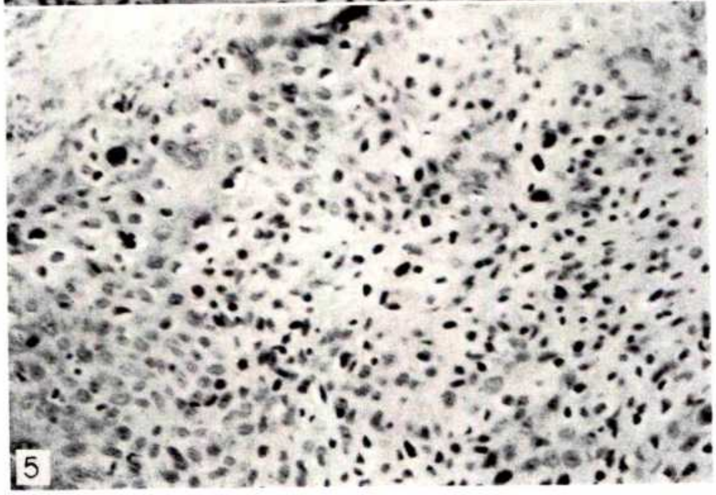


FIGURE 4. Dysplastic cells of bowenoid area clearly shown (H & E, × 200).

FIGURE 5. Complete loss of architectural organization and bowenoid transformation (H & E, × 200).

loma shows areas of beginning disruption of the architecture of the lesion with bowenoid changes (Fig. 2). More centrally the loss of direction of growth of the cells and the appearance of a 'wind-swept' vista are more appreciable (Fig. 3). At higher power the large and dysplastic cells are evident (Fig. 4). Another field at high power (Fig. 5) shows the loss of orderly growth pattern and bowenoid presentation of the papilloma.

DISCUSSION

Prior to the distinction of actinic (senile) keratosis from S.V. by Freudenthal (1926) and Pinkus (1958) among others, there was some confusion about the possibility of S.V. showing squamous dysplasia. Becker (1951) has a good discussion of this point. Since the separation of actinic keratosis from S.V., it appears that all the effort of the investigators has been concentrated on the emphasis of this fact and has ignored the occasional dysplastic changes of S.V. into bowenoid growth. For example, Rowe (1957) stated that: 'No unequivocal evidence was found, to indicate that seborrheic keratoses ever give rise to actual cancer.' As all the progressive effort has been in the direction of extrication of S.V. from the pre-cancerous actinic keratosis group, any evidence that may have indicated that S.V. could also be pre-cancerous has either been discarded or not noted. Only a few authors have documented their observations of this possibility. Civatte (1954) recounted the case of a 60-year-old man who had developed a verrucous lesion over the years which on clinical examination showed a typical S.V. with no elements of inflammation. But on histological viewing Civatte (1954) gave the description of a papillomatous growth with areas of bowenoid degeneration. Christeler & Delacretaz (1966) described a clear-cut case of seborrhoeic verruca with malignant transformation of bowenoid type.

Kwittken (1974) presented fourteen cases of S.V. with areas of *in situ* carcinoma-like changes. All, except one of his cases showed signs of recent inflammation and some of these *in situ* carcinoma-like changes could have been what one sees in traumatized and inflamed S.V. Still, one of his cases (case 13) could truly fit the lesions presented here as S.V. with bowenoid transformation. He also presented five cases of superficial basal cell epitheliomas (S.BCE) which he felt arose from S.V. These could have represented S.BCE with acanthosis of the overlying epidermis, or concomitant occurrence of S.BCE and S.V., as the two lesions seemed to coexist with no admixture of growths.

The cases presented here showed that, although S.V. is a benign epithelial growth, there is the possibility of bowenoid dysplasia and presumably the chance of development of locally invasive lesions and eventual possible distant metastasis in a very small number of cases of S.V. Personally no such cases were seen and the appearance of bowenoid changes will not necessarily be followed by the above possibilities. This paper is presented to document this histopathological observation in a small percentage of seborrhoeic verrucae.

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ADDENDUM

Since the submission of this paper for publication, there has been another case presentation of this interesting bowenoid transformation of seborrhoeic verruca by P.H. Bloch (*Journal of Cutaneous Pathology*, 1978, 5, 361).

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Aggressive basal cell carcinoma in Nigerians

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SUMMARY

It is important to emphasize that basal cell carcinoma occurs in black Africans, a fact denied in some reports. The present study shows that the disease appears to behave more aggressively in the African, particularly in albinos, than in Caucasians. In albinos preventive measures should be employed regularly.

We have defined aggressive basal cell carcinoma as one whose behaviour defies surgical or other methods of therapy. This defiance may be manifest either in relentless and uncontrollable local tissue invasion by tumour or in the establishment of distant metastasis. In a recent study from Ibadan, Itayemi & Oluwasanmi (1974) found two cases of basal cell carcinoma with lymph node metastasis in a small series of nineteen cases of basal cell carcinoma in black Nigerians; similar cases of metastasizing basal cell carcinoma have occasionally been published (Lattes & Kessler 1951; Raitschev, Andreev & Stojanov, 1961; Singer, 1944; Constanza *et al.*, 1974). In addition, we have encountered a few cases of basal cell carcinoma uncontrollable by local resection. Altogether six cases of aggressive basal cell carcinoma were found in this study. The study period was 10 years from 1966 to 1975.

CASE REPORTS

Case 1

A.A.S. Hosp. No. 178715 is a 35-year-old female albino with nodular basal cell carcinoma of the inner canthus of the right eye. The duration of the lesion was 1 year prior to presentation here. Radical surgery including orbital exenteration and excision of the right half of the nose has not cured the lesion. When last seen, an area under the left ala of the nose showed induration, indicating neoplastic spread in this direction. The patient was referred elsewhere for radiotherapy.

Case 2

J.U. Hosp. No. 176155. A 45-year-old black male Nigerian with a basal cell lesion of the scalp of 3 years duration (Fig. 1). Excision was followed within 1 year by advanced bilateral cervical lymph node

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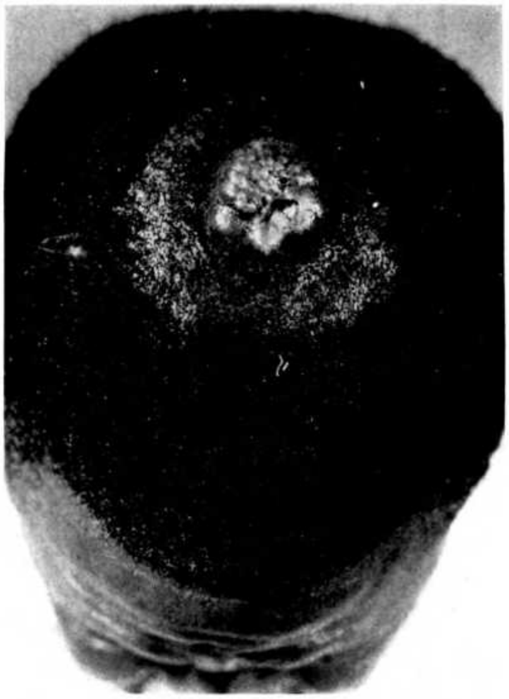

FIGURE 1. Basal cell carcinoma of the scalp.

secondaries histologically confirmed as basal cell carcinoma. He was treated with methotrexate, but died soon afterwards—within a few months of discharge from hospital.

Case 3

P.O. Hosp. No. 332656. A 50-year-old female albino had multiple deep and extensive basal cell carcinomas of the left shoulder, forehead, back, left cheek and nose. The left shoulder joint was exposed; there was involvement of the skull, and the posterior ribs. The patient had trismus due to tumour invasion of the cheek muscles. The disease was too advanced for surgical therapy. She was sent home on analgesics.

Case 4

D.A. Hosp. No. 226713. A 60-year-old black female Nigerian with a lesion situated between the two breasts, in the central part of the lower chest. There were bilateral enlarged axillary lymph nodes and lumps in both breasts. Biopsy of the chest lesion showed a basal cell carcinoma. She was treated with methotrexate with no response and disappeared from the clinic within 3 months. No lymph node biopsy was done.

Case 5

S.O. Hosp. No. 290263. A 60-year-old male Nigerian, again with a lesion in the central part of the chest which was radiologically shown to have extended into the anterior mediastinum. The lesion was of 10 months duration and cannon ball secondaries were seen on radiographs of the lungs. Biopsy of the chest lesion confirmed basal cell carcinoma. He was discharged on analgesics.

Case 6

D.A. Hosp. No. 73512. A 30-year-old male Nigerian albino who underwent resection of a basal cell carcinoma of the face. Two years later he developed cervical lymph node metastases. Radical neck dissection was done and histological examination again confirmed basal cell carcinoma in the excised lymph nodes.

PATHOLOGY

The specimens received in the cases reported varied in size, ranging from a few millimetres to a few centimetres in diameter. Some of the gross specimens had a raised pearly border with central depression. Others showed central ulceration, as well as crusting. However, the specimen of lymph node received in Case 6 weighed 140 g, was greyish in colour, and measured 9 × 9 × 4 cm.

The formalin fixed paraffin embedded sections were stained with haematoxylin and eosin and examined under the light microscope. The histology of the skin lesion was diagnostic in all our cases. The characteristic cell of basal cell epithelioma contains a large blue-staining nucleus with minimal cytoplasm, giving the tumour a basophilic appearance. The tumour cells at the periphery of any nest or lobule of tumour tissue are arranged with their long axis perpendicular to the edge of that lobule. This lining up of cells gives a pallisading appearance to the periphery. Characteristically, mitotic figures are seen only occasionally in basal cell carcinoma. However, Cases 2 and 6 showed a total replacement of the lymph nodes by garlands of metastatic basal cell carcinoma with a high mitotic index.

DISCUSSION

Although Beadles (1874) gave the first description of metastasizing basal cell carcinoma, Jackson & Adams (1973) described horror basal cell carcinoma and gave four criteria:

(a) size—large size of greater than 3 cm diameter;
(b) destruction of deep and essential structures;
(c) local uncontrollability;
(d) metastasis.

The first three factors are not always easy to separate from one another, and in Nigeria where patients are often late in seeking treatment, the size of the lesion is not direct evidence of aggressiveness; rather it may indicate the delay between the onset of the lesion and the patient's first attendance. Hence we have used local uncontrollability, in spite of 'adequate' treatment, and distant (metastatic) spread as the two main criteria of aggressiveness.

In a study of skin tumours in the Nigerian, Oluwasanmi, Alli & Williams (1969) found that basal cell carcinoma occurred in a disproportionately high number of albino Nigerians (28%) in a population where albinos were less than 1% of the total population. In the present study, it is of interest that of six cases fitting the description of aggressive basal cell carcinoma, half were in albinos. This number of aggressive basal cell carcinomas is disproportionately high over a period in which the total number of basal cell carcinomas was twenty-two.

Two of the cases with cervical lymph node secondaries satisfied the criteria laid down by Cotran (1961) for a diagnosis of metastatic basal cell carcinoma:

(1) The primary tumour must arise from the skin.
(2) Metastasis should not be explicable on the basis of direct extension from the primary.
(3) The histology of both primary and metastatic lesions should be similar.

With the histological picture of Cases 2 and 6, we are convinced that we are dealing with aggressive basal cell carcinoma that has metastasized rather than locally infiltrated these nodes.

It is interesting that some writers (Fritz, 1964) have doubted the fact that basal cell carcinomas occur in the black African. It is rather surprising that our small series of basal cell carcinomas should contain so many aggressive lesions. Issues raised by this high percentage of aggressive basal cell carcinomas in Africans include the nature of the tumour and the effectiveness of host defences.

The aggressive lesions have not been shown to be a particular morphological or clinical type. The histology of the lesion here is very similar to those seen in Caucasian or other races elsewhere. It may be that the local or generalized host resistance is lower in Africans. This is well seen in albinos among whom basal cell carcinoma is common even in early life—a phenomenon not seen among normally pigmented individuals. It appears that in susceptible people, the heavy actinic irradiation does locally diminish their response to skin cancer, and may therefore abet tumour aggressiveness.

If heavy actinic irradiation does produce a more malignant outcome to an otherwise relatively benign growth, due thought should be given to the treatment of these lesions in Africans. Not only should local excision be virtually as radical as in squamous cell carcinoma; the question of prevention must be accorded the importance it deserves. Protective measures such as the use of light barrier creams and sun shades, and working indoors, may improve the health and the quality of life particularly in susceptible people like the albino.

Many cases of basal cell carcinoma are treated less radically than is usual for cancers, e.g. by curettage (Reymann, 1975) or by the use of irritants such as podophyllin. It would be interesting to find out whether any of these methods of treatment have been followed by metastasis. A long follow-up is essential as secondary deposits may occur many years after the primary lesion (Binkley & Rauschkolb, 1962). In view of the great percentage of aggressive lesions, such methods of therapy are not recommended for basal cell carcinoma in the Africans.

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We wish to thank Mr Enyogai of the Medical Illustration Unit for the photograph.

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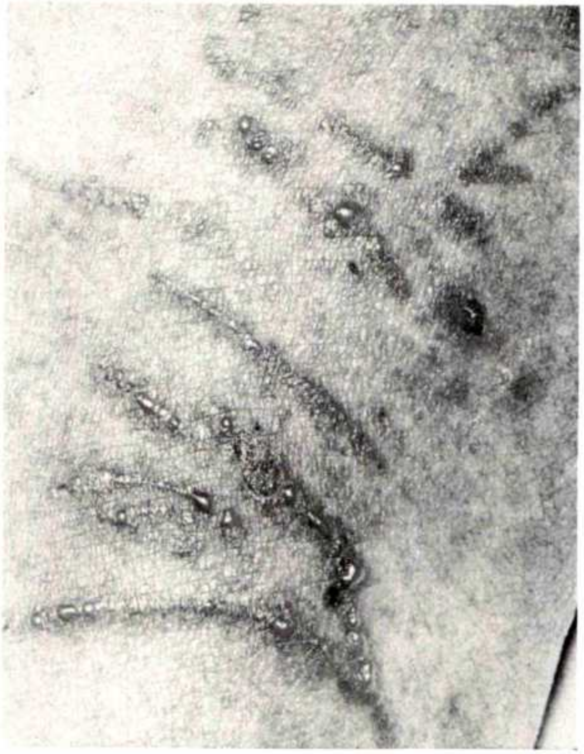

FIGURE 2. Patient 2. Linear excoriations and blisters on right forearm. The patient was on predisone 10 mg daily and had been taking corticosteroids for 6 weeks. At the time of this photograph no other skin lesions were present.

rash. Numerous clear tense blisters arising from an erythematous base were present on the limbs and trunk. Several linear blisters were noted beneath excoriations of the epidermis (Fig. 3). The clinical diagnosis was juvenile pemphigoid. Histology showed a subepidermal blister consistent with pemphigoid. Direct immunofluorescence demonstrated linear IgA (but not IgG) deposition along the basement membrane with weak staining for C3. Circulating IgA basement membrane antibody in a maximum titre of 1:80 was repeatedly demonstrated by indirect immunofluorescence using guinea-pig lip substrate. Indirect immunoelectronmicroscopy (Professor K. Holubar, Vienna) demonstrated IgA deposition in the lamina lucida in a pattern similar to IgG deposition in typical pemphigoid (Schaumberg-Lever *et al.*, 1975; Holubar *et al.*, 1975).

DISCUSSION

We suspect that initiation of lesions by minor trauma may not be a very uncommon feature in pemphigoid. However, because it is usually only a minor aspect of the disease it is likely to be overlooked. Occasionally, however, trauma-induced lesions may dominate the clinical picture as at one stage in Patient 2.

In both Patients 1 and 2 lesions could be produced either experimentally or 'spontaneously' during the first 2–6 weeks of systemic corticosteroid treatment and at a time when other lesions were healing or totally suppressed. Both of these patients had typical histological and immunological features of

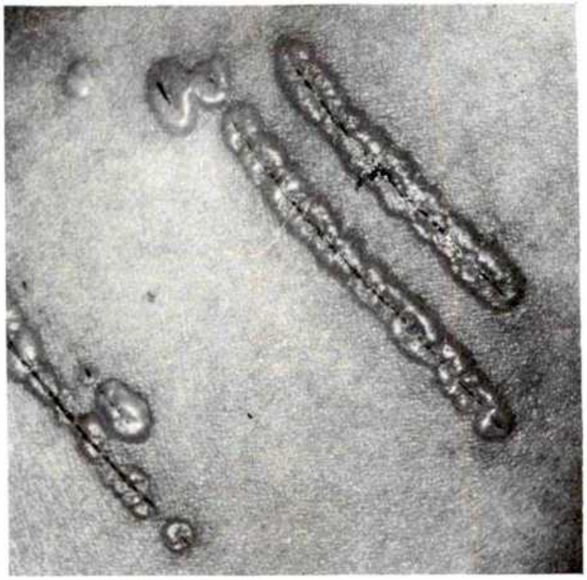

FIGURE 3. Patient 3. Linear blisters on left thigh. Linear excoriations of the epidermis are clearly visible in the blister tops.

pemphigoid even though in Patient 2 these developed fully some weeks after the onset of clinical lesions.

Patient 3, with juvenile pemphigoid, was unusual in that the basement membrane antibody was exclusively of IgA class. She closely resembled the patients described by Esterley *et al.* (1977) with a diagnosis of chronic bullous dermatosis of childhood. However an additional finding in our patient was the presence of circulating basement membrane IgA antibody, a feature recently described by others (van der Meer *et al.*, 1977; Pehamberger, Konrad & Holubar, 1977; Yaoita & Katz, 1977). While the classification of such patients is at present controversial we favour the diagnosis of pemphigoid on the basis of the clinical and histological findings, the presence of *in vivo* bound and circulating antibodies against skin basement membrane giving a typical pemphigoid pattern on immunofluorescence and immunoelectron microscopy.

Clearly in the majority of patients with pemphigoid, trauma-induced lesions are not of clinical importance. There is a close analogy with psoriasis in which trauma-induced lesions (Köbner phenomenon) may often be found on careful search but rarely dominate the clinical picture. However, we believe that failure to recognize this aspect of the disease in pemphigoid may sometimes lead to diagnostic difficulty.

A review elsewhere in this issue of recently published reports of patients with epidermolysis bullosa acquisita suggests that this disease may represent a variant of pemphigoid in which trauma is the predominant initiating factor for the development of lesions (Dahl, 1979).

In addition to the trauma-induced lesions on the gums (Forman & Nally, 1977), Cram & Fukuyama (1972) have described the induction of pemphigoid lesions by another physical agent—ultraviolet irradiation. Cutaneous lesions of allergic angiitis may be induced by cold or the application of suction (Copeman & Ryan, 1971) or by intradermal injection of vasoactive substances such as histamine (Braverman & Yen, 1975; Gower *et al.*, 1977) or by trauma (Soter *et al.*, 1978). All these stimuli, including direct trauma and UV irradiation, may act through the common pathway of increased vascular permeability and in allergic angiitis there is evidence that this results in immune complex deposition (Braverman & Yen, 1975; Gower *et al.*, 1977). Circulating immune complexes have been detected in pemphigoid (Tappenheiner *et al.*, 1977) and the dermal histology of pemphigoid lesions frequently shows a perivascular infiltrate of neutrophils and nuclear dust (Lever & Schaumburg-Lever, 1975) similar to leukocytoclastic angiitis. Further investigations using physical and pharmacological stimuli to initiate lesions may prove useful in studying the immunopathogenesis of pemphigoid.

ACKNOWLEDGMENTS

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Comment

EPIDERMOLYSIS BULLOSA ACQUISITA—A SIGN OF CICATRICIAL PEMPHIGOID?

Epidermolysis bullosa acquisita (EBA) is a doubtful entity according to Rook (1972). He suggested that many patients with EBA were in fact suffering from epidermal necrolysis or porphyria cutanea tarda. In the most recent review of EBA (Roenigk, Ryan & Bergfeld, 1971) many previous case reports were rejected and four criteria on which a diagnosis of EBA should be based were suggested. These criteria were as follows: (1) Clinical lesions of epidermolysis bullosa dystrophica: mild trauma producing bullae over joints of hands, feet, elbows and knees; atrophic scars; milia; and nail dystrophy; (2) adult onset of disease; (3) negative family history for epidermolysis bullosa; and (4) other bullous diseases excluded: porphyria cutanea tarda, normal urinary uroporphyrins; pemphigus, histology and immunofluorescence (direct and indirect); pemphigoid, histology and immunofluorescence (direct and indirect); dermatitis herpetiformis, histology and immunofluorescence (direct and indirect); and bullous drug dermatitis. These criteria have been repeatedly quoted in subsequent case reports of EBA. However none of the three patients reported by Roenigk *et al.* (1971) fully conformed to their own criteria since direct immunofluorescence was not performed on the skin of any of them and in only two was the serum examined by indirect immunofluorescence.

Subsequently there have been case reports of patients with a diagnosis of EBA which have included direct immunofluorescence studies of the skin. In all these patients, immunoreactants at the basement membrane zone (BMZ) have been demonstrated and the resemblance to pemphigoid has been noted frequently. However this diagnosis has been dismissed because of the clinical findings and negative indirect immunofluorescence. It is the purpose of this paper to review recent observations on EBA and to indicate the strong clinical, histological and immunological similarities which exist between EBA and pemphigoid, particularly cicatricial pemphigoid (CP).

CASE REPORTS

The data presented are taken from nine case reports of EBA published between 1973 and 1978 which have included direct immunofluorescence studies of the skin. A further patient is included with a diagnosis of ? epidermolysis bullosa who closely resembled the others.

Clinical features

The clinical details of these patients are shown in Table 1.

The age of onset of the disease ranged from childhood to 69 years and there were seven males and three females. All except one had had their disease for over 1 year and one for approximately 40 years. None had a family history of blistering disease. Associated conditions included Crohn's disease in four, one of whom also had psoriasis, and pulmonary tuberculosis, pulmonary fibrosis and cryoglobulinaemia in one each. Atrophy and scarring at the sites of lesions were present in all patients and milia were recorded in nine. Hyper- and/or hypopigmentation at the sites of lesions was recorded in four patients. The knuckles and dorsa of the hands were involved in all patients and bony prominences such as the elbows and knees were affected in most. Lesions on the trunk were observed in four

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TABLE 1. Clinical details of patients with EBA

Patient	Source	Sex	Age at onset (years) Duration of disease (years)	Sites of lesions: Skin	Sites of lesions: Mucosal	Sites of lesions: Nail dystrophy	Other conditions/response to treatment
1	Kushniruk (1973)	F	69 8	Hands, elbows, knees feet, buttocks, lower legs	Tongue, lips	Fingers, toes	Responded to prednisone 80 mg →20 mg daily
2	Kushniruk (1973)	M	26 ½	Knuckles, elbows, ankles	Gingivae		Crohn's disease No response to topical corticosteroids
3	Gibbs & Minus (1975)	F	66 8	Hands, feet, trunk	Tongue	Fingers, toes	Responded to prednisone 60 mg →0 daily
4	Metz, Metz & Frank (1975)	M	22 2	Hands, elbows, knees, feet	Palate, conjunctivae	Fingers, toes	Crohn's disease. No response to systemic corticosteroids
5	Fayolle *et al.* (1975)	M	42 3	Hands, elbows, knees, legs, feet, trunk, neck, face	Tongue, buccal mucosa, conjunctivae	Fingers, toes	Active pulmonary tuberculosis
6	Tan (1977)	M	67 1½	Hands, elbows, knees, feet		Fingers, toes	Pulmonary fibrosis
7	Goodwin & Eady (1977)*	M	childhood 40 (approx.)	Lower legs, hands, trunk		Toes	Controlled on 60 mg prednisone daily but relapsed on 40 mg daily—no response to azathioprine
8	Cheesbrough & Kinmont (1978)	F	24 6	Hands, elbows			Crohn's disease, psoriasis
9	Livden *et al.* (1978)	M	18 1	Hands, elbows, knees, face, neck	Tongue, lips, buccal mucosa, gingivae	Fingers, toes	Crohn's disease—slight response to prednisone and azathioprine
10	Krivo & Miller (1978)	M	59 1	Hands, elbows, knees			IgG–IgM cryoglobulinaemia

*Author's diagnosis—? epidermolysis bullosa.

patients and on the face in two. Mucosal lesions were reported in six patients including involvement of the tongue (4), lips (2), gingivae (2), buccal mucosa (2), conjunctivae (2) and palate (1). The conjunctival lesions consisted of non-specific conjunctivitis in Patient 4 and symblepharon in Patient 5. Nail dystrophy involved fingers and toes in five patients and toes only in another. The presence of scarring around the nail beds was described in Patients 1 and 3.

Immunological investigations

The results are shown in Table 2.

On direct immunofluorescence examination of the skin, BMZ deposition of IgG was found in all ten patients. Other immunoreactants detected at the BMZ were C3 (8/8), fibrin/fibrinogen (3/4), IgM (2/6), IgA (1/5—oral mucosa only) and C4 (1/1). In six reports the authors comment on the similarity of the immunofluorescent pattern to that of pemphigoid, but this diagnosis was rejected on clinical grounds. In four patients the immunofluorescent pattern was described as granular.

TABLE 2. Immunoreactants detected by immunofluorescence at skin basement membrane

	Direct immunofluorescence						
Patient	IgG	IgM	IgA	C3	C4	Fibrin/ fibrinogen	Indirect immunofluorescence
1	+	—	—	+	ND	ND	—
2	+	—	—	+	ND	ND	—
3	+	ND	ND	ND	ND	ND	—
4	+	+	—	+	ND	ND	—
5	+	ND	ND	+	ND	ND	ND
6	+	ND	ND	+*	ND	ND	—
7	+	ND	ND	ND	ND	+	—
8	+	—	ND	+	ND	+	—
9	+	—	+†	+	ND	+	—
10	+	+	—	+	+	—	—
Total positive	10/10	2/6	1/5	8/8	1/1	3/4	0/9

* Reported as 'complement'.
† Oral mucosa only.
ND—no data.

In most reports the type of skin examined by immunofluorescence was not clearly stated. In these cases it appears that only paralesional specimens were examined. However in Patient 4 BMZ deposition of IgG, IgM and C3 was found only in paralesional skin and was not detected in clinically normal abdominal skin. By contrast Patient 9 showed linear BMZ IgG and C3 in clinically normal skin from various sites. In Patient 10, who had an IgG–IgM cryoglobulinaemia, fine granular deposits of IgM were seen. C3 and C4 were noted in small dermal blood vessels of paralesional skin.

Serum from nine of the patients was tested by indirect immunofluorescence for antibodies to skin BMZ with uniformly negative results.

Light microscopy

Table 3 shows the histological features of patients with EBA.

Subepidermal blistering with dermo-epidermal separation was present in all patients. PAS staining was performed in seven and splitting of PAS-positive basement membrane which adhered partly to the roof and partly to the floor of the blister was noted in six of these. In seven patients the presence of a dermal inflammatory cell infiltrate was recorded although in one this was described as not significant. The infiltrate was described as perivascular in three patients and eosinophils were recorded in three together with neutrophils in two. In the remaining cases terms such as 'mild inflammation', 'round cell' and 'lymphohistiocytic' were used.

TABLE 3. Histological features of biopsies from patients with EBA

		Dermal cell infiltrate			
Patient	Basement membrane*	Perivascular	Neutrophils	Eosinophils	Other
1	Split	Yes	ND	ND	Round cell
2	Blister roof	Yes	ND	ND	Round cell
3	Split (early lesion)	ND	ND	ND	ND
	Blister roof (old lesion)	ND	ND	ND	ND
4	Split	Yes	Yes	Yes	Lymphocytes, histiocytes
5†	ND	Yes	ND	Yes	Lymphocytes, histiocytes
6	Split	ND	ND	ND	Not significant
7	ND	ND	Yes	Yes	ND
8	ND	ND	ND	ND	Mild chronic inflammation
9	Split	ND	ND	ND	Mild inflammation
10	Split	ND	ND	ND	Vasculitis not observed

* PAS staining.

† Multiple biopsies—variable intensity of inflammatory infiltrate; some bullae contained eosinophils.

ND—no data.

Electron microscopy

In four patients lesional skin was examined by electron microscopy. In Patient 7 the blister was formed in the lamina lucida between the plasma membrane of the epidermal basal cells and the basal lamina. In Patients 3, 4 and 5 the blister formed beneath the basal lamina. Fine granular non-cellular material was found beneath the basal lamina in all three of these patients. In two patients (3 and 4) this material formed a continuous band immediately beneath the basal lamina separating it from the dermis. In these two patients this amorphous material adhered to the basal lamina to form the roof of the blister. In Patient 5, finely granular material was noted in the upper dermis but appears to have been in discrete deposits rather than as a continuous band and was not associated with blister formation. The ultrastructural appearances of this material suggested amyloid but histochemical stains for amyloid were negative and its nature was not determined in any of the three patients.

Response to treatment

Systemic corticosteroids satisfactorily controlled blistering in two patients (Patients 1 and 3). In Patient 7 control of blisters was achieved only with an unacceptably high dose and in two others (Patients 4 and 9) there was little or no response to systemic corticosteroids although the dose was not stated. Azathioprine, either alone or in conjunction with corticosteroids in these two patients, appears to have had little effect. No other satisfactory treatment was reported in any of the patients.

DISCUSSION

The findings on direct immunofluorescence of the skin were very similar to those of pemphigoid. IgG, which was present at the BMZ in all the patients with EBA, is the commonest immunoglobulin class found in the skin in bullous pemphigoid (BP) (Jordan, Triftshauser & Schroeter, 1971) and CP (Bean *et al.*, 1972; Holubar, Honigsmann & Wolff, 1973; Michel *et al.*, 1977). Other immunoglobulin classes including IgM and IgA may sometimes be found in BP (Jordan *et al.*, 1971; Provost & Tomasi, 1974) and in CP (Holubar *et al.*, 1973; Griffith *et al.*, 1974; Michel *et al.*, 1977; Rogers *et al.*, 1977). In the patients with EBA, IgM was found in addition to IgG in the skin of two patients and IgA in the oral mucosa of another. Complement C3 is also commonly found at the BMZ in both BP (Ahmed, Maize & Provost, 1977) and CP (Bean *et al.*, 1972; Holubar, *et al.*, 1973; Griffith *et al.*, 1974) and was present in all the patients with EBA who were investigated. C4 was detected in one patient and has also been found in CP (Holubar *et al.*, 1973). Linear deposition of fibrin which was found in three of four patients with EBA is also a common finding in CP (Rogers *et al.*, 1977).

In BP, immunoreactants bound to the BMZ can often be detected in non-lesional skin at any site as was the case in Patient 9 of this series. However in some patients, particularly those with localized disease, immunoreactants may be detectable only in perilesional skin. This was the case in Patient 4 with EBA.

The pattern of BMZ immunofluorescence was usually linear and in six of the patients a diagnosis of pemphigoid was considered. A granular fluorescent pattern was reported in three. In two of these the biopsy specimens contained blisters which, in the author's experience, may disrupt the pattern of fluorescence in BP immediately adjacent to the blister presumably due to early damage to the BMZ. A fluorescent pattern of this type is illustrated in the report of Patient 10. In Patient 9, the published immunofluorescence photomicrograph appeared typical of pemphigoid although the authors rejected this diagnosis on the basis of invalid arguments (Dahl, 1979). In summary, both the type and pattern of immunoreactant found at the BMZ in these patients with EBA are entirely consistent with the findings in BP and CP.

Circulating immune complexes (IgG–IgM cryoglobulin) were found in Patient 10 in whom deposits of IgM, C3 and C4 were also detected in small dermal blood vessels. Circulating immune complexes are frequently found in BP (Tappenheimer *et al.*, 1977) and may also be found in many of the diseases which have been associated with both pemphigoid and EBA (*vide infra*). Several authors argued that the absence of circulating anti-BMZ antibodies was evidence against a diagnosis of pemphigoid. However, even in active BP such antibodies are not always demonstrable and were found in only twenty-nine of thirty-six (81%) patients in a recent series (Ahmed *et al.*, 1977). Although circulating antibodies have occasionally been found in CP (Dantzig, 1973; Bean, 1974; Dabelsteen *et al.*, 1974; Tagami & Imamura, 1974), they are uncommon. In fifty-seven patients with CP, circulating antibodies were found in only three (Person & Rogers, 1977a). Absence of circulating antibodies has been reported in other patients with EBA (Roenigk *et al.*, 1971, Cases 1 and 3; Almeyda 1972; Pegum & Wright, 1973; Reed *et al.*, 1975, Case 3). However it is clear that the absence of circulating antibodies in no way excludes a diagnosis of pemphigoid and negative findings predominate in CP.

Subepidermal blister formation with histological separation at the dermo–epidermal junction is of course consistent with both BP and CP as well as other blistering diseases. Splitting of the PAS-positive basement membrane was first described in EBA by Epstein, Epstein & Greenlee (1959). It is a feature which has been frequently quoted since then in connection with EBA and was found in six of seven patients in the present series. However similar basement membrane splitting is also found in CP (Susi & Shklar, 1971).

The dermal changes in CP are non-specific (Lever & Schaemburg-Lever, 1975). Eosinophils are less prominent in CP than in BP (Person & Rogers, 1977a) and may be absent (Behlen & Mackay, 1965). Eosinophils were specifically mentioned in the dermal infiltrate in three of the ten patients with EBA and in two of these, neutrophils were also present. The histological features in these patients with EBA were therefore quite similar to those described in CP and a diagnosis of pemphigoid was suggested by the histopathologist in Patient 5.

The ultrastructural sites of blister formation in the four patients were not consistent. In this respect they resemble the findings in both BP and CP. In BP the basal lamina has been variously reported to be situated in the blister roof (Pierard & Kint, 1968), in the blister base (Braun-Falco & Rupec, 1967; Jacubowicz, Dabrowski & Maciejewski, 1970) and to be destroyed in the process of blister formation (Schaumburg-Lever, Orfanos & Lever, 1972). In CP the basal lamina has been found partly in the roof and partly in the base of oral blisters (Susi & Shklar, 1971), wholly at the blister base or more usually destroyed within blisters (Caputo, Bellone & Crosti, 1973).

In all three EBA patients in whom blisters formed beneath the basal lamina fine granular material of unknown nature was observed beneath the basal lamina. Rather similar material was described by Caputo *et al.* (1973) in CP and a granular thickening of the basal lamina observed in relation to oral lesions of CP (Susi & Shklar, 1971). The nature of this material and whether it is the same in CP and EBA remain to be determined. However there seem to be ultrastructural similarities between EBA and CP and certainly no features which clearly distinguish the two.

Discussion of the clinical features of these patients has been left until last because it was mainly on the basis of the clinical features that a diagnosis of pemphigoid was rejected by several of the authors. Roenigk *et al.* (1971) reported a wide age range in EBA (9–75 years) which was very similar to the present series. Although the majority of patients with both BP and CP are over 60 years old (Person & Rogers, 1977a) BP in young adults and children is well recognized and CP may also occur at a younger age (McCarthy & Shklar, 1958). Roenigk *et al.* (1971) reported a preponderance of males in EBA (eleven of seventeen patients) and in the present series there was also a preponderance of men (seven of ten patients). This differs from the accepted equal sex ratio in BP and the excess of females usually reported in CP (Person & Rogers, 1977a). However if, as is suggested, EBA is part of the spectrum of CP, it might be anticipated that men, who tend to be more exposed to trauma, might show this aspect of the condition more frequently than women.

The clinical features of atrophy, scarring, milia and pigmentary changes are all consistent with CP as is the involvement of mucosal surfaces which was observed in six of the ten patients. Non-specific conjunctivitis is typical in early ocular involvement (Person & Rogers, 1977a) while symblepharon is a characteristic late feature. As has been pointed out by Dantzig (1974) the prevalence of ocular involvement varies widely between 20% and 100% in different series of CP. Both the type and prevalence (20%) of ocular disease in these patients with EBA are consistent with CP.

One of the main objections to a diagnosis of pemphigoid has been the clear association of lesions with physical trauma. However other physical agents have been shown to induce pemphigoid lesions including ultraviolet irradiation (Cram & Fukuyama, 1972). Suction blister time is reduced in BP and correlates with disease activity (Comaish & McVittie, 1973). In some patients with BP, 'spontaneous' lesions may clearly be induced by the trauma of scratching (Dahl & Cook, 1979). A mechano-bullous disorder of the gingivae which could be induced by mild trauma has also been reported (Forman & Nally, 1977). They considered that this was a variant of CP and, in two out of five patients investigated, immunoreactants (C3 in both, IgG in one) were found at the BMZ or oral mucosa. There is, therefore, considerable clinical and experimental evidence to indicate that the induction of lesions by trauma in EBA does not exclude a diagnosis of pemphigoid.

The final clinical feature which weighed heavily against a diagnosis of pemphigoid was the presence of nail dystrophy. As far as the author is aware nail dystrophy has not been associated with CP. However, if the foregoing evidence is accepted, that BP and CP lesions may be induced by trauma, then it follows that localization of lesions at sites susceptible to trauma such as the fingers and bony prominences is to be anticipated. If lesions produce scarring it follows that nail dystrophy is a logical outcome. Although there is no mention of nail dystrophy in the text, the report by Dantzig (1973) of a patient with CP contains a colour illustration of the hands which shows not only nail dystrophy, but also hyperpigmentation and scarring on the backs of the fingers almost identical to the illustration in the report of Patient 3 with EBA in this series. The particular significance of Dantzig's patient was that not only was linear BMZ deposition of IgG and C3 demonstrated in the skin, but also this was the first patient with CP in whom circulating anti-BMZ antibodies were also demonstrated. Perhaps awareness of the possible association of nail dystrophy with CP may stimulate reporting of further cases.

The association of EBA with Crohn's disease in this series is remarkable. Previous reports (Roenigk *et al.*, 1971; Pegum & Wright, 1973) suggest that this association is not a chance one. Many patients with EBA have other systemic diseases (Roenigk *et al.*, 1971) as may patients with pemphigoid (see Person & Rogers, 1976 for references). In view of the finding of circulating immune complexes in pemphigoid (Tappenheiner *et al.*, 1977) it is of interest that circulating immune complexes have been found in many of the diseases which have been associated with both pemphigoid and EBA. These include malignancy (Teshima *et al.*, 1977), rheumatoid arthritis (Winchester, Kunkel & Agnello, 1971; Luthra *et al.*, 1975; Zubler *et al.*, 1976b), systemic lupus erythematosus (Agnello *et al.*, 1971; Zubler *et al.*, 1976a), inflammatory bowel disease (Doe, Booth & Brown, 1973; Jewel & MacLennan, 1973; Hodgson, Potter & Jewel, 1977; Nielsen *et al.*, 1978) and diabetes mellitus (Irvine *et al.*, 1977).

Although CP can be controlled with systemic corticosteroids and other immunosuppressive drugs, it is generally considered to be less responsive to corticosteroids than BP (Person & Rogers, 1977a). The poor response to systemic corticosteroids in three of five patients with EBA is consistent with this feature of CP. BP is sometimes responsive to dapsone (Person & Rogers, 1977b) and one of the patients with CP reported by Michel *et al.*, 1977 was controlled with sulphapyridine. A patient with EBA who responded to dapsone and other sulphonamides has been reported (Purdy & Fairbrother, 1972).

In summary, many of the clinical, histological, electron microscopical and immunological features of EBA are consistent with pemphigoid and particularly with CP. Some other features such as traumatic induction of lesions and nail dystrophy are not so inconsistent with a diagnosis of CP as might first appear. The present evidence continues to suggest that EBA is not a disease entity. If the term is to be retained, perhaps it should be used to describe a particular group of clinical features and physical signs, i.e. an acquired subepidermal blistering disease induced by trauma and resulting in scarring. In that case EBA would be a feature of porphyria cutanea tarda, CP and perhaps other conditions.

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Book Reviews

Dermatology in Internal Medicine. By SAM SHUSTER (1978). Oxford: Oxford University Press. Pp. 290. Price £5.95.

This is a fascinating book but a book difficult to review. Those who know the author will not be surprised by some of his views. Many will enjoy learning that he is now prepared to admit that changes in the skin can occur as a result of internal disease although he stresses, quite rightly, that on the whole, the skin and the internal organs are independent. It might be that within the next 20 years we will find that he admits that occasionally a dermatological condition can be precipitated by emotional (psychological) disturbance and that the association is not always the other way round as is emphasized in this book.

There is a very comprehensive and well written account of the skin manifestations of a whole range of internal diseases arranged in logical groups. When known, the underlying causative mechanisms are concisely discussed and few will disagree with these. Some would not yet go along with him in grouping benign mucous membrane pemphigoid as a variant of bullous pemphigoid. Such are minor details, as are the occasional small errors in the text and in the bibliography.

Those dermatologists who lecture on their subject to 'Advanced Medicine Courses' or MRCP classes will find a mine of easily assimilable information on the present state of knowledge of the changes produced in the skin by the different disease processes. These changes are well recognized by an experienced dermatologist but it is not for such that the book is written. The author in his preface poses the question 'So for whom other than myself was I writing?'. The reply he gives 'Mostly for the postgraduate with an interest: either enforced by the MRCP examination, for which this should do the trick, or spontaneous, when the cure might take longer'. He extends this to include the new trainees in dermatology. However, there is not a single picture to illustrate any of the lesions which he describes. An illustrative but not exhaustive list is given of various types of drug reaction but the non-dermatologist will find this almost incomprehensible.

Dermatologists will enjoy reading this book both for the enormous amount of fact it contains and for the pleasure of riding Sam's particular hobby horses. It cannot be recommended for its intended readership in the absence of a good atlas or a comprehensive series of slides. The non-dermatologist trainee who has access to either of these will probably not also need the book.

RENWICK VICKERS

Cancer Dermatology. Edited by FREDERICK HELM (1979). Philadelphia: Lea and Febiger. Pp. 501, 6 col. plates. Price £31.50.

This textbook is a multi-author production with the contributors coming mainly from the eastern seaboard of the United States. It is divided into three sections: a short contribution on the aetiology neoplasms of skin, a long descriptive section on clinical aspects of skin malignancy, and lastly a fairly extensive discussion on the management of skin cancer.

The section on the aetiology of neoplasms of the skin is brief and includes short contributions on epidemiology, carcinogenesis, an interesting contribution on epithelioma resulting from single trauma, and an excellent chapter on genodermatoses associated with malignancies. The point is made in the section on epidemiology that at the present time we are sadly lacking in good epidemiological information on non-melanoma skin cancer, a point which must surely be rapidly corrected if current worries about the use of ultraviolet irradiation with or without the use of photosensitizing agents and its association with future risk of malignancy is ever to be established.

The section on neoplasms of the skin is divided into chapters describing various specific malignancies and also such situations as precancerous lesions and pseudo-malignancies. The section on cutaneous markers of internal malignancy is extremely comprehensive and perhaps rather uncritical. It is suggested that while the evidence for pemphigoid in association with malignancy is lacking it is proven for pemphigus vulgaris. This particular text is also confident that adult dermatomyositis has a significant association with malignancy. The section on appendage tumours is rather brief and I personally would have liked to have seen more discussion in the section on precancerous lesions which include such conditions as extra-mammary Paget's, lichen sclerosus et atrophicus, balanitis xerotica obliterans and giant pigmented naevi.

The section devoted to the management of skin cancer covers surgical procedures, irradiation, cautery, the use of the cryosurgery, Mohs' chemosurgery, chemotherapy and immunotherapy. It is a section which dermatologists on this side of the Atlantic are not likely to read in depth but perhaps this is something which should be changed in British dermatology.

This text at present probably has more to offer the trainee in clinical oncology than the trainee dermatologist, but nevertheless at £31.50 it is a useful and worthwhile addition to the literature. It is profusely illustrated with black and white prints and six colour plates are found in the section on malignant melanoma.

RONA M.MACKIE

Physical Modalities in Dermatologic Therapy. Radiotherapy, Electrosurgery, Phototherapy, Cryosurgery. Edited by HERBERT GOLDSCHMIDT. Berlin: Springer-Verlag. Pp. 290+XV, 301 B/W, 16 col. illustrations, 62 tables. Price DM 87, U.S. $47.90.

This hard back, 300 page book, edited by Herbert Goldschmidt, consists of twenty-six chapters, fifteen of which concern the radiotherapy treatment of skin conditions. The other chapters cover such topics as electrocautery, electrosurgery, phototherapy, iontophoresis, ultrasound, laser surgery and the plasma torch. Dr Goldschmidt has edited the previous book on skin radiotherapy written by Dr Braun-Falco, and once more, the early chapters on radio-biology, the physical aspects of radiotherapy and radiation and cell kinetics are dealt with lucidly, with clear diagrams. However, the subsequent increasing detail with which the various clinical aspects of skin tumour radiotherapy are dealt with gets out of hand.

For a dermatologist who has never used radiotherapy, the basic diagrams, photographs of multiple types of eye shields and copious repetitive photographs of skin masking, would I am sure prove a detailed guide. However, in this country, radiotherapy is generally used only by dermatologists or radiotherapists well grounded in the basic aspects of this speciality, and they do not conduct the treatments themselves. Therefore the indication for such detail is difficult to understand, and would be more appropriate to a radiographers textbook. It enlarges the book disproportionally, and may be dangerous in suggesting that radiotherapy is a treatment form akin to others, which may be used by the occasional practitioner.

The book is therefore dedicated to the American market and such practices as treating plantar warts by radiotherapy and treating basal cell epitheliomas by Grenz rays are not common British practice. Many dermatologists will find the extensive chapters on other physical methods of treatment unnecessary in the context of the hospital clinic in England. There is an attempt to treat each subject minutely, explaining not only the basic principles behind the treatment, but also giving many photographs of the range of apparatus available in a field. This will unfortunately result in the book becoming rapidly outdated. The many beautifully produced photographs and diagrams eventually become an encumberance and the diagram of how to kill two rabbits by strychnine and cyanide poisoning respectively, with iontophoresis, seemed, like some other diagrams, unnecessary.

I do not think that British dermatologists or radiotherapists will rush to buy this book, not only because it costs $47.90, but because the welter of detail of differing forms of treatment is more appropriate to the American clinic practice than to the British practice of either dermatology or radiotherapy.

M.F.SPITTLE

Pathogenic Streptococci. Edited by M.T.Parker (1979) Chertsey: Reed Books. Pp. 294. Price £21.00.

This book-of-the-symposium carries in brief form the proceedings of the VII International Symposium on Streptococci and Streptococcal Disease held in Oxford in September 1978. That an attractive, well illustrated book set in print can be produced in so short a time is a tribute to the contributors, but more especially to the editor and the publisher, though the price cannot be a matter of congratulation for something as ephermeral as conference proceedings. There is much here to interest the dermatologist or those otherwise interested in skin. Papers on the immune response, on glomerulonephritis following pyoderma, and on the recently recognized meat-associated skin infection will be of immediate interest. Immunization against *Strep. mutans* to protect against dental caries will provoke thought as will the emergence of a 'new' pathogen in the perinatal infection due to group B beta haemolytic streptococci and the suggestion that viridans streptococci in bacteraemia may be of skin origin. The microbiologically fascinating study of taxonomy may not stimulate the clinician but more precise definition contributes to our knoweldge of the epidemiology of disease and ultimately to its prevention. The format of the book encourages repeated dipping for new snippets of knowledge; it will be a boon for those seeking an overview of the medical importance of streptococci.

W.C.Noble

Probleme der Erythrozytopoese, Granulozytopoese und des Malignen Melanoms. Edited by G.W.Lohr, H.Arnold, R.Engelhardt, W.Mobius, G.Mahr, F.Schmalzl and Ch.Sauter (1978) Berlin: Springer Verlag. Pp. 457, 205 B/W, 12 col. illustrations. Price DM 98.

This publication is the 21st volume in the series 'Hämatologie und Bluttransfusion' and comprises the proceedings of two conferences held in 1976. The first meeting was the annual congress of the German Society for Haematology held in Freiburg and the topics covered include iron metabolism, drug-induced anaemias and malignant melanoma. The section on iron metabolism is up-to-date and well-referenced and includes contributions on iron deficiency anaemias and iron storage diseases such as haemachromatosis. The section on drug-induced anaemias is shorter and rather more selective. Of greater interest to dermatologists will be the section on malignant melanomas. This commences with an article by Dr Heite on clinical and prognostic aspects of malignant melanoma. Dr Heite has had extensive experience in coordinating the German co-operative melanoma study and between the years 1962 and 1972 has accumulated data on some 2,600 cases of malignant melanoma. The review is good but covers relatively little new ground. Dr Hornstein's contribution on histological classification and assessment of malignant melanomas is well written and informative. In addition to reviewing well-known histological prognostic factors such as level of invasion and tumour thickness he discusses the significance of other factors such as the degree and type of tumour vascularization in relation to prognosis. Short contributions on the immunotherapy, chemotherapy and radiotherapy of malignant melanoma are all adequately covered by experts in these fields.

The second section of the book consists of the proceedings of a meeting of the Austrian Haematological Society and includes twenty-one papers with the themes of functional disturbances of non-leukaemic leukocytes and the immunotherapy of malignant disorders of haemopoiesis. Many of these papers would not be of primary interest to general dermatologists but there are interesting short contributions on disturbances of neutrophil and monocyte function.

One would not envisage that many dermatologists would wish to buy this book, which has a strong haematological bias and includes a number of unrelated topics. However, the sections on malignant melanoma and iron metabolism in particular would be useful reading for those wishing to keep up-to-date in these fields.

Neil Smith

Frontiers of Matrix Biology. Vol. 6. I. Elastases/ Structure, Function and Pathological Role. II. Cyclic AMP and the Skin. Edited by J.Bieth, G.M.Collin-Lapinet and L.Robert. Basel: S.Karger. Pp. 210, 54 illustrations, 31 tables. Price U.S. $62.00.

This volume is in two separate sections—I. Elastases: Structure, Function and Pathological Role; and II. Cyclic AMP and the Skin. The former would be of minimal interest to the dermatologist being a very detailed description of various elastases including sequencing and comparison with similar proteases.

The second half consists of seven chapters by various authors which follow a standard pattern of a general introduction followed by detailed experimental results and data acquired by the authors in different fields. A mass of detail is presented which is difficult to sort out. It is, however, very well documented. Unfortunately, the indexing does not live up to the extensive bibliography. Its chief value would be to shorten any literature search on cyclic AMP and the skin.

C.N.D.Cruickshank

Aktuelle Probleme der Medizinischen Mykologie. Vol. 3 of the Series: Hygiene für die Praxis. By Waltraud Braun (1978) Berlin: Volk und Gesundheit. Pp. 88, 24 illustrations, 7 tables. Price DM 10.30.

This slim volume I found to be very discursive and it undoubtedly attempts to compress far too many aspects of medical mycology into too small a space. It ranges from the development of the subject through ecology, classification, diagnosis, clinical conditions, serology, mycotoxins and at times verges on the philosophical. Certainly not to be recommended for the casual reader of German.

C.N.D.Cruickshank

British Journal of Dermatology (1979) **101**, 489.

News and Notices

British Society for Dermatopathology, Winter Meeting, January 1980

The annual meeting of the Society will be held on the afternoon of Thursday, 17 January and the morning of Friday, 18 January 1980. As last year the meeting will immediately precede that of the Investigative Group of the British Association of Dermatologists.

On 17 January there will be a symposium on autoimmune disease held at the Anderson College Building, 56 Dumbarton Road, Glasgow G11 6NU. The guest speaker will be Professor Agr. J.H. Saurat on 'Graft versus host reaction'.

On the morning of 18 January there will be a clinicopathological demonstration of cases at the Royal College of Physicians and Surgeons of Glasgow, 234–242 St Vincent Street, Glasgow G2 5RJ.

Members of the Society will be circulated with further details in due course. Enquiries regarding the meeting, Society membership, etc., should be addressed either to Dr F.M.McGregor, Department of Pathology, Royal Infirmary, Glasgow G4 0SF, or to Dr D.H.McGibbon, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

Seminar

A one-day multi-disciplinary national seminar on 'Chronic skin conditions, the National Health Service, the patient and the community' will be held on Wednesday, 14 November 1979 at: The London Hospital Medical College, The London Hospital, Turner Street, Whitechapel, London EC1 2AD.

This will, through Speakers and Syndicate Sessions, seek to identify the many problems arising in respect of these conditions in the family, employment, social and medical situations, and make recommendations as to how they can be resolved.

Application forms and full details can be obtained from: Miss Margaret Dowden, Conference Secretary, The Disabled Living Foundation, 346 Kensington High Street, London W14 8NS. Tel. 01 602 2491.

Appointments

South Glamorgan Area Health Authority (Teaching)

Dr Peter J.A.Holt has been appointed Consultant Dermatologist to the South Glamorgan Area Health Authority. Dr Holt graduated M.B., Ch.B. from Sheffield University Medical School in 1969 and obtained his M.R.C.P. in 1972. Following general medical posts in Sheffield and Cardiff he was appointed Registrar and later Senior Registrar in Dermatology at Cardiff. In 1976, he spent a year working as Lecturer in the Department of Dermatology at the University of Oklahoma, Oklahoma City, U.S.A. His special research interests and publications include the investigation of the effects of endocrine hormones on the epidermis, and the study of cell surface glycoproteins in diseases of the epidermis. He has also written several papers on subjects of clinical interest.

Contact Dermatitis Investigation Unit, Glasgow

Dr Angela Forsyth has been appointed Consultant at the Contact Dermatitis Investigation Unit based in Belvidere Hospital, Glasgow.

She qualified M.B., Ch.B. from Glasgow, and gained M.R.C.P. U.K. after a medical rotational post. Dermatology had always been her main interest, and she entered this specialty in 1973. Since then, while completing the required training programme, she gained wide clinical and teaching experience both as Registrar in Stobhill Hospital, Glasgow and as Senior Registrar in the Western Infirmary, Glasgow.

Dr Forsyth developed an interest in Contact Dermatitis, and spent 6 weeks at the department in Glasgow during her training. More recently she spent 4 weeks at the Contact Clinic at St John's, Lisle Street, London.

Research interests have been mainly clinical, including the skin of diabetics, skin changes while using topical antimitotic cream, and contact dermatitis in children.

Cambridgeshire Area Health Authority (Teaching)

Richard J.Pye has been appointed to the post of Consultant Dermatologist at Addenbrooke's Hospital, Cambridge. He was educated at Ardingly College and qualified at Charing Cross Hospital in 1969. Prior to specializing in Dermatology, he obtained wide experience in acute general medicine at the Charing Cross group of hospitals, and the United Norwich Hospitals. He became a member of the Royal College of Physicians in 1973. In 1974 he became Registrar to the Dermatology Department at Bristol, and as a result of research work carried out there, was awarded his M.D. (London). He then gained further experience as Senior Registrar at St John's Hospital, London.

He has published a large number of papers on clinical and investigative dermatology. His main research interest has been in the physiology and pathology of the sebaceous glands.

South Western Area Health Authority

Dr David J.Gould has been appointed Consultant Dermatologist to the Cornwall Area Health Authority based at the Royal Cornwall Hospital, Truro.

He graduated M.B., Ch.B. Hons. (Leeds) in 1972 and became M.R.C.P. in 1975. Dr Gould trained in general medicine at The General Infirmary, Leeds and Neath Hospital, South Wales. He was Senior House Officer and Registrar in the Department of Dermatology, Leeds and later Lecturer in the University Department of Dermatology, Sheffield. His main investigative work has been on leukocyte function in acne vulgaris. He has published work on the treatment of psoriasis with methotrexate and a number of papers of clinical interest.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R.H.Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations,* A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper on feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editors(s); (2) year of publication; (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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MORPHOLOGICAL DERMATOLOGY: A Study of the Living Gross Pathology of the Skin *by* **Robert Jackson,** *Ottawa Civic Hospital, Ottawa, Ontario, Canada. Foreword by* **Barney Usher.** Emphasizing morphological differential diagnosis, this text, in each of its twenty-six chapters, details a particular morphological lesion along with its various forms, method of examination, and diagnostic approach. Erythema, urticaria, purpuras, telangiectases, eczemas, bullous dermatoses, papules, nodules, ulcers, keratoses, dyschromias, cutaneous atrophies and dystrophies, onychoses, cysts, and burrows are among the many specific lesion groups explored. A thorough glossary and an appendix of surface anatomical region names complement the text. *'79, 400 pp. (6 3/4 x 9 3/4), 88 il., 5 tables, $32.50*

CHEMOSURGERY: Microscopically Controlled Surgery for Skin Cancer *by* **Frederic E. Mohs,** *Univ. of Wisconsin Medical School, Madison, Wisconsin.* Written by the originator and developer of chemosurgical techniques, this volume comprehensively explains methods for the microscopically controlled excision of cancers of the face, mouth, sinuses, larynx, parotid gland, penis, scrotum, and other accessible structures. Practical information is also provided on the chemosurgical treatment of noncancerous growths. *'78, 400 pp. (6 3/4 x 9 3/4), 834 il., 59 tables, $43.75*

SKIN SURGERY (4th Ed.) *edited by* **Ervin Epstein** *and* **Ervin Epstein, Jr.,** *both of Univ. of California Medical School. (45 Contributors)* Prominent authorities in such fields as dermatology, surgery, and plastic surgery have combined their expertise to produce this extensively illustrated text on all aspects of skin surgery. Divided into eight sections, the text covers general considerations, cold steel surgery, electrosurgery, special procedures, special locations of disease, chemosurgery, cryosurgery, and investigative surgical therapy of the skin. *Plastic and Reconstructive Surgery* lauded the text as "superbly organized, clearly written, and beautifully illustrated." *'77, 864 pp. (7 x 10), 1081 il. (19 in color), 37 tables, $113.75*

DERMABRASION AND CHEMICAL PEELING: In the Treatment of Certain Cosmetic Defects and Diseases of the Skin *by* **James W. Burks.** *Foreword by* **Morris Waisman.** This well-illustrated treatise on the clinical application of dermabrasion is based on the author's performance of over 25,000 such operations. Chapters cover anatomy of the skin, histopathology of the peeling process, effect of freezing on the skin, surgical technique, and psychological considerations. Other chapters describe equipment, anesthesia, postoperative care, complications, and future trends. Approximately two hundred photographs illustrate every step of the procedure as well as day-by-day and before-and-after views of patients dermabraded for various reasons. *'79, 256 pp., 192 il., 5 tables, $22.50*

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CRYOSURGICAL ADVANCES IN DERMATOLOGY AND TUMORS OF THE HEAD AND NECK *edited by* **Setrag A. Zacarian,** *Boston Univ., Boston, Massachusetts. Forewords by* **John G. Bellows** *and* **Edward A. Krull.** *(11 Contributors)* This book explores the fundamentals of cryogenesis, the development of the cryolesion, temperature monitoring, double freeze-thaw cycles, and the pathogenesis of cryonecrosis. Cryosurgery for treatment of acne and specific cutaneous neoplasia is discussed along with other applications of this extremely effective procedure. *'77, 296 pp. (7 x 10), 473 il. (122 in color), 31 tables, $38.00*

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[1]Hanna N F, Clay, J C, Harris, J R W, Br J Vener Dis 1978, 54, 354
[2]Syntex Pharmaceuticals Limited Data on file *DERBAC is a trademark

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Textbook
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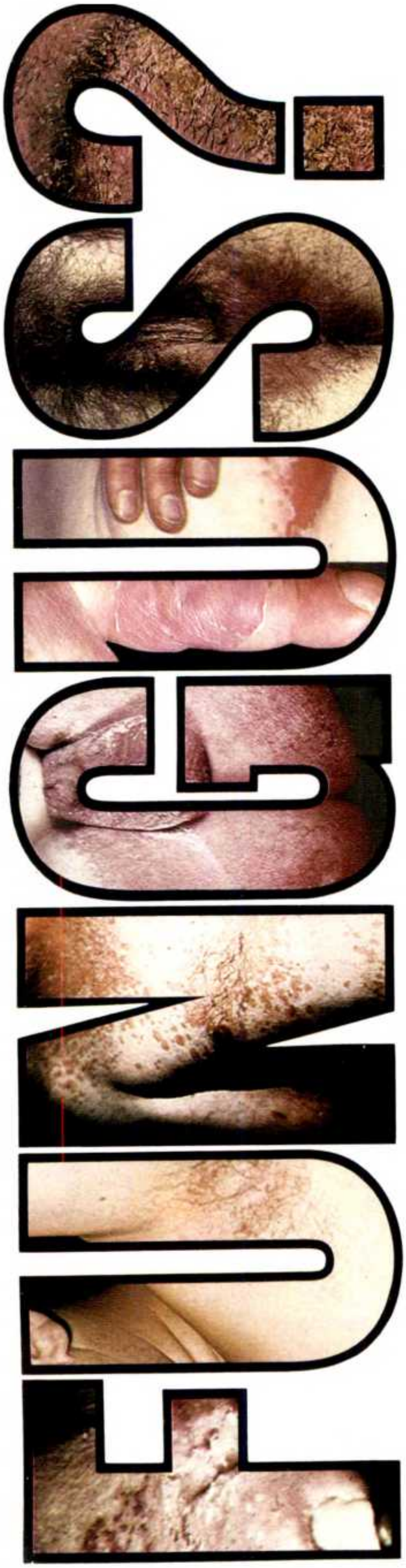

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1978. 112 pages. Paper, £2.00

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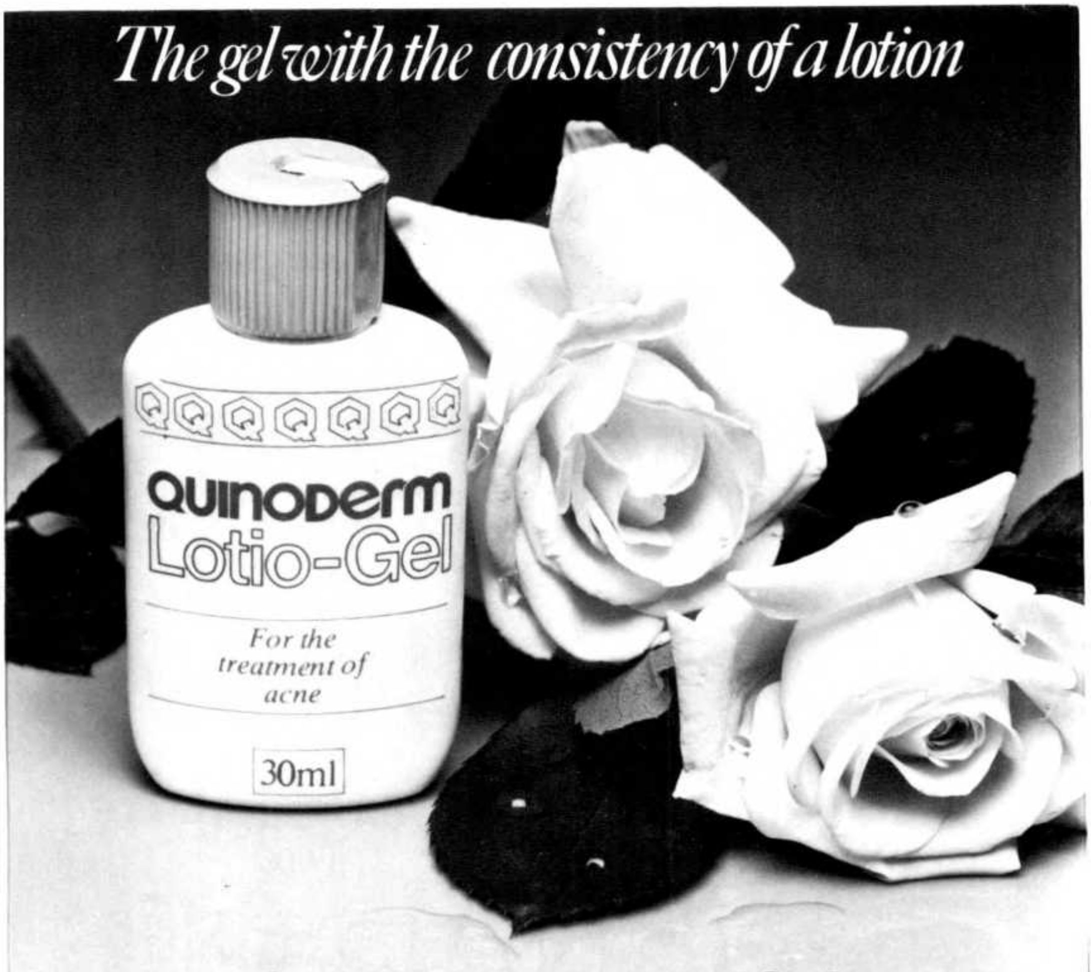
New Product Introduction



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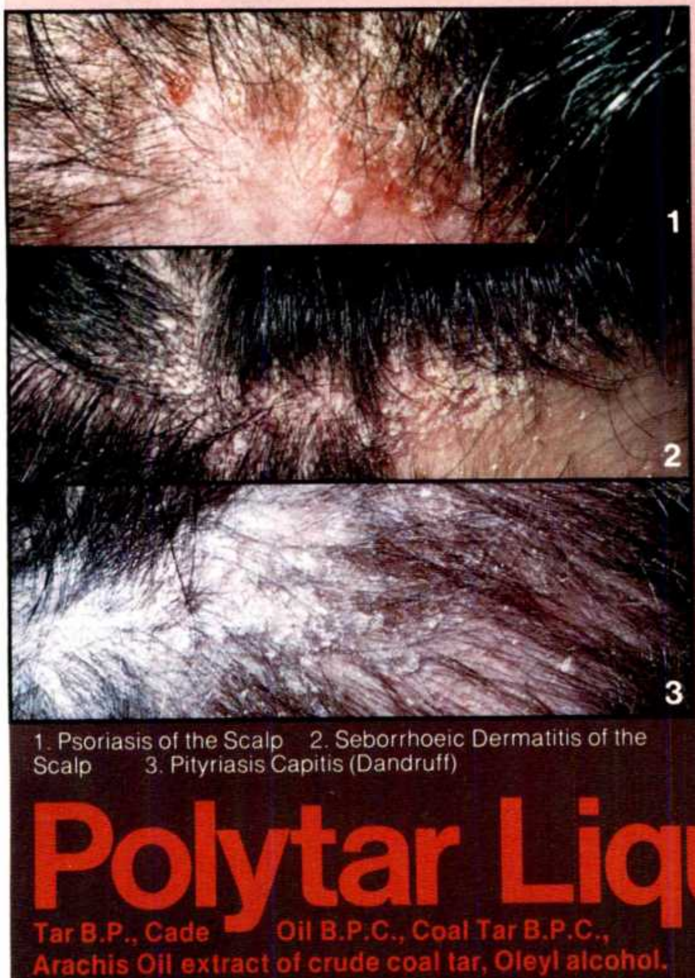
Common Disorders of the Scalp.



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Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
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Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

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Product Licence Number
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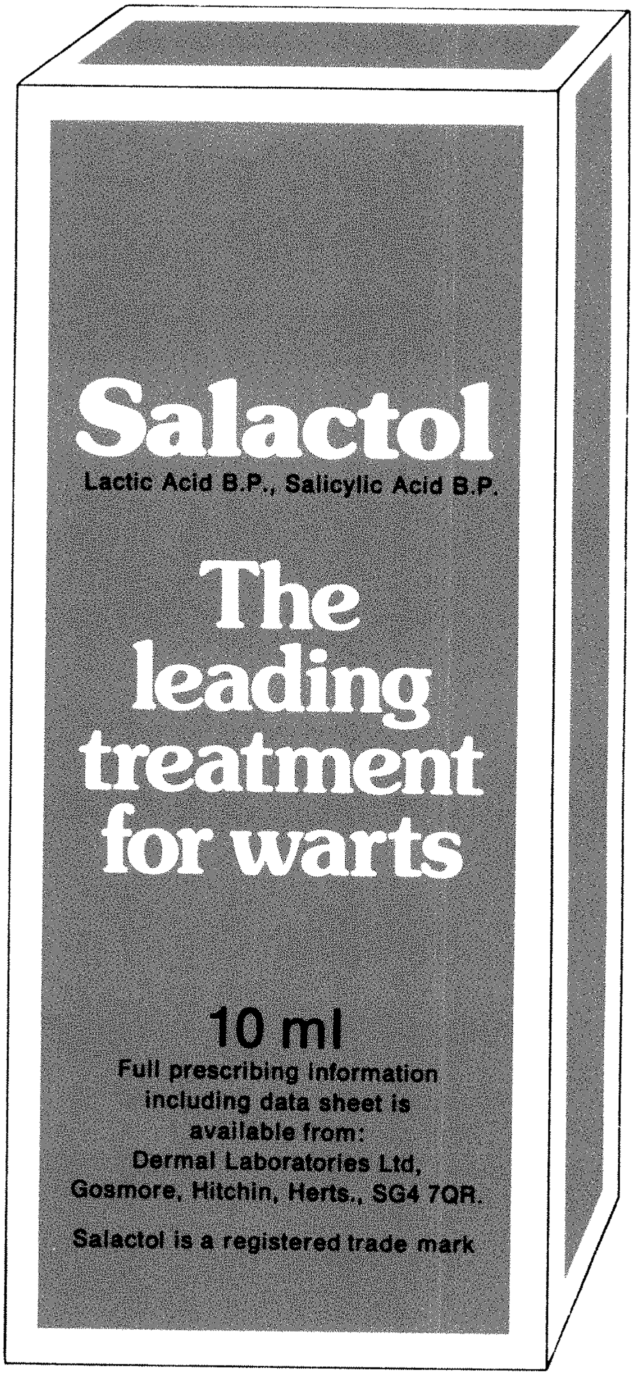
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British Journal of Dermatology (1979) **101**, 617.

Clinical and Laboratory Investigations

Studies on poison ivy

IN VITRO LYMPHOCYTE TRANSFORMATION BY URUSHIOL–PROTEIN CONJUGATES

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Accepted for publication 18 March 1979

SUMMARY

The isolation and purification of poison ivy urushiol is described. The preparation of urushiol–ski protein and urushiol human serum albumin is also described. Lymphocytes from eleven dono naturally sensitized to poison ivy and from four non-sensitive individuals have been cultured fo 5 days in the presence of urushiol–carrier conjugates. Lymphocytes from seven of the eleven sensitiv donors responded with a stimulation index greater than 3·0 to urushiol–albumin conjugate. When urushiol–skin protein conjugate was used as a stimulant, lymphocytes from only three of the eleven sensitive donors responded. The results suggest that urushiol–protein conjugates can stimulate sensitive lymphocytes *in vitro*, although a response is not observed in every individual naturally sensitized to poison ivy.

Contact dermatitis to poison ivy is frequently encountered in North America (Fisher, 1973). The agent responsible for the allergic reactions has been identified as a mixture of 3-n-pentadecylcatechol and three similar catechols possessing varying degrees of olefinic unsaturation in the side chain (Dawson, 1956; Kligman, 1958) (Fig. 1). The di-olefin derivative III is the most active allergen (Johnson *et al.*, 1972).

The exact mechanism by which these compounds can induce contact dermatitis is not fully understood. It has been proposed that oxidation of the catechol to the *ortho*-quinone derivative is a necessary step in the pathogenesis of poison ivy dermatitis. The quinone derivatives can then react with a suitable carrier by a Michael-type (nucleophilic) addition (Mason & Lada, 1954; Byck & Dawson, 1968).

The present study describes isolation of the active principle (urushiol) of the poison ivy plant, the preparation of urushiol–protein conjugates and a study of the ability of these conjugates to induce lymphocyte transformation *in vitro* in patients naturally sensitized to poison ivy and in non-sensitive individuals.

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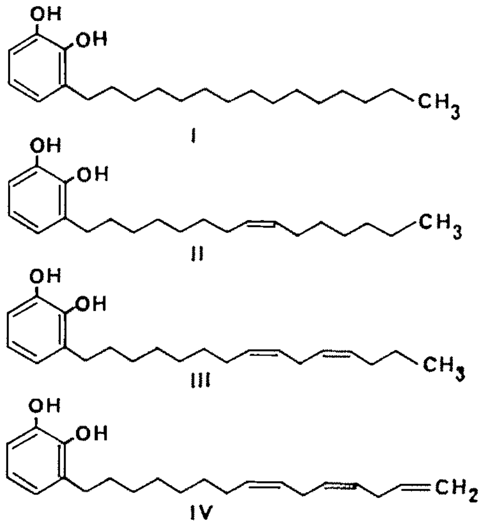

FIGURE 1. Chemical structures of the constituents of poison ivy urushiol: 3-n-pentadecylcatechol (I), 3-n-(pentadecenyl-8′)-catechol (II), 3-n-(pentadecadienyl-8′,11′)-catechol (III) and 3-n-(pentadecatrienyl-8′,11′,14′)-catechol (IV).

MATERIALS AND METHODS

Patients

Fifteen lymphocyte donors, 25–45 years of age, participated in this study. The donors were divided into two groups: sensitive patients (eleven, all male) and non-sensitive (control) patients (two males, two females). Sensitive individuals were selected on the basis of documented sensitivity to poison ivy. Last recorded exposure ranged from 1 week to 3 years.

Urushiol isolation

Freshly collected plants (*Rhus radicans*, family Anacardiaceae) were collected in the Laurentian area, north of Montreal, in mid-July. Some plants had already started to bear fruit. The fresh material (5 kg) was ground in a Waring blender with ethanol (20 l). The ground material was pressed through cheese-cloth and the green alcoholic solution was evaporated to a viscous dark syrup at 45°C *in vacuo*. The syrup was redissolved in ethanol and the suspension filtered. Silicic acid 100 mesh (15 g, Mallinckrodt, St Louis, Missouri) was added to a portion (approximately one-half) of this solution and the mixture was evaporated to dryness *in vacuo*. The urushiol-impregnated silicic acid was placed on top of a column (5×30 cm) containing silicic acid suspended in purified petroleum ether (60–100°C). The column was eluted with petroleum ether (500 ml) and with 5% ethyl ether/95% petroleum ether. Fractions of 20 ml were collected and the desired material was located by the $FeCl_3/Na_2CO_3$ test (Hill, Mattacotti & Graham, 1934). These fractions were pooled, evaporated *in vacuo*, dissolved in ethyl acetate and applied to a column (5×50 cm) of polivinylpyrrolidone (PVP Type NP K-30, General Aniline and Film Corp., New York, U.S.A.) which was eluted with ethyl acetate. Individual fractions (20 ml) were spotted on a t.l.c. plate (silica gel G) which was developed in 30% ethyl acetate/70% petroleum ether. Urushiol was visualized by spraying diazotized paranitroaniline, followed by a solution of sodium carbonate. A total yield of 16 g of a slightly coloured oil was obtained; R_f 0·6 (silica gel G, ethyl acetate-petroleum ether, 3:7), retention time of the diacetyl derivative by g.l.c. (Fig. 2), 20·5 min (single peak) (1% SE-30 on acid-washed chromosorb W, 60–80 mesh: column dimensions, ¼ inch × 6 feet, injector temperature, 230°C; detector temperature, 240°C; elution at 220°C with nitrogen, 60 ml/min).

Purification of human serum albumin

Human serum albumin (HSA) (Connaught Laboratories, Toronto, Ontario) was purified by chromatography on Sephadex G-200 using 1% sodium chloride as eluant. The front fraction which represents aggregates was discarded. The fraction containing the desired protein was collected, dialysed (H_2O) and lyophilized.

Preparation of skin protein extract

Human skin specimens (8 g) were obtained from autopsies. The fresh tissue was scraped free of fat and hair and washed in saline. The skin was then ground in the presence of cold (−20°C) acetone and filtered. The process was repeated until the filtrate was colourless. The tissue was dried in a dessicator *in vacuo*. The dried powder was extracted with 0·067 M phosphate buffer, pH 7·4 (100 ml) for 2 h at room temperature. The extract was centrifuged, dialysed against the same buffer (4 l) at 4°C, concentrated by filtration on an Diaflo membranes PM 10 (Amicon Corporation, Lexington, Massachussetts) and chromatographed on a Sephadex G-200 column (2 × 120 cm) equilibrated with 1% sodium chloride. The major protein fraction was rechromatographed on the same column. The final fraction (42 mg) showed one major band on polyacrylamide gel electrophoresis at pH 9·3, with minor contaminants.

Preparation of urushiol–protein conjugates

(*a*) *With human serum albumin.* A solution containing oxidized urushiol (5 mg) (Byck & Dawson, 1968) in dioxane (1 ml) was added over a period of 1 h to a solution of human serum albumin (50 mg) and Brij (125 mg) (Fisher Scientific, Montreal) in 0·067 M phosphate buffer pH 6·8 (25 ml). The solution was stirred at 4°C overnight. The mixture was concentrated on a Diaflo PM 10 membrane and chromatographed twice on a Bio-Gel P-100 column (1·5 × 90 cm) equilibrated with 1% sodium chloride. The purified conjugate was sterilized by filtration on a 0·22 μm Millipore membrane (Millipore, Mississauga, Ontario).

(*b*) *With skin proteins.* The urushiol–skin protein conjugate was prepared essentially as described above, using oxidized urushiol (5 mg) in dioxane (1 ml), Brij 35 (100 mg) and purified skin proteins (20 mg) in 0·067 M phosphate buffer pH 6·8 (20 ml). The conjugate was purified as described above.

Protein concentration was determined by the procedure of Lowry *et al.* (1951) using bovine serum albumin (Sigma Chemical Co., St Louis, Missouri) as standard.

Lymphocyte cultures

Lymphocytes were obtained by venipuncture and cultured as described (Dupuis & Brisson, 1976) at a concentration of 1×10^6 cells/ml (total volume, 2 ml/culture tube) for a period of 5 days. ^{3}H-thymidine incorporation was used as index of lymphocyte stimulation.

RESULTS

Urushiol prepared by the technique described here gives a fraction which appears to be relatively free of other contaminants by chromatography on thin layer plates and by gas chromatography

(Fig. 2). The use of a polivinyl-pyrrolidone column greatly helps to free the desired material of contaminating pigments and chlorophyll. Oxidation of urushiol by treatment with $AgNO_3$ gave a material which could be readily coupled to human serum albumin and to skin proteins. Comparative ultraviolet spectra showed that the peaks observed at 274 nm (skin proteins) or at 277 nm (human serum albumin) were less defined (shoulders) after reaction of the proteins with oxidized urushiol (Fig. 3).

Table 1 shows the results of ^{3}H-thymidine uptake by lymphocytes from individuals with a history of sensitivity to poison ivy, and from control individuals. Various concentrations of proteins and urushiol–protein conjugates were used in order to assess their effect on lymphocyte stimulation. With the lymphocytes from donors sensitive to poison ivy, maximum stimulation was dependent on the concentration of conjugates used in cultures. The results are more apparent when the stimulation index, i.e. the ratio of ^{3}H-thymidine incorporation by lymphocytes in the presence of the conjugate over the carrier alone, is calculated (Table 2). In the case of non-sensitive donors, the stimulation index is in the range 0·5–3·1. If an arbitrary value of 3·0 is chosen as an indicator of sensitivity, it can be seen that lymphocytes from seven of the eleven sensitive donors show a stimulation index greater than 3·0.

Urushiol–skin protein conjugate appears to be a weak stimulant. At a concentration of 1 μg/ml, three (L.L., J.C. and C.P.) of the eleven sensitive donors gave a response, whereas only two of those responded to a concentration of 2·5 μg/ml. Urushiol–albumin conjugate stimulated lymphocytes from five of the eleven sensitive donors at a concentration of 10 μg/ml, four at a concentration of 25 μg/ml and only three at 37·5 μg/ml.

Lymphocytes from donors A.P., P.B., Y.B. and D.T. were not stimulated by the conjugates. M.L. responded only to a high concentration (37·5 μg/ml) of urushiol HSA conjugate. Patients L.L.,

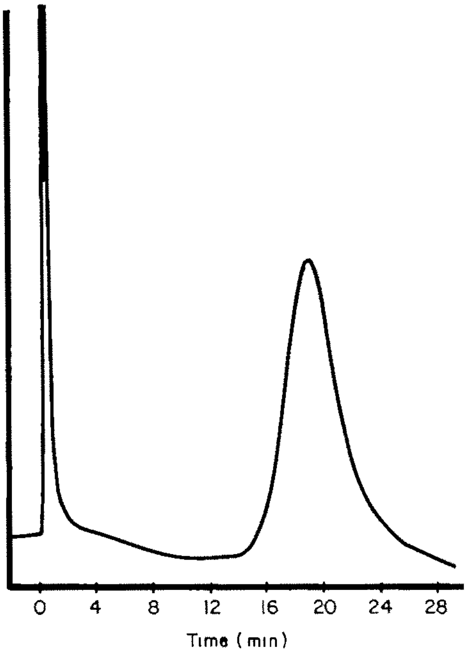

FIGURE 2. Gas chromatographic analysis of purified urushiol. Details are described in the text.

TABLE 1. ³H-thymidine incorporation (DPM) by lymphocytes cultured in the presence of human serum albumin or purified skin proteins and their corresponding urushiol conjugates

		DPM														
		Sensitive donors											Control donors			
Stimulant	Quantity (µg/ml)	M.L.	A.P	P.B.	R.L.	Y.B.	F.G.	L.L.	J.C.	J.B.	C.P.	D.T.	H.N.	B.R.	R.L.	D.G.
S.P.	1	—	40700 (14800)	—	—	1600 (170)	1620 (520)	970 (120)	1640 (50)	1500 (180)	670 (120)	4800 (1040)	—	—	—	—
S.P.	2·5	5600 (900)	16400 (7600)	3920 (1600)	1380 (270)	1350 (160)	920 (140)	1020 (180)	2340 (950)	1400 (230)	660 (60)	6300 (2400)	6200 (1560)	2230 (430)	1070 (140)	4850 (110)
URS–S.P.	1	9640 (1300)	32000 (8800)	5240 (250)	2710 (130)	2650 (1090)	1940	13730 (250)	8200 (2800)	2100 (2300)	3080 (800)	5000 (580)	5240 (1000)	1760 (1000)	— (160)	—
URS–S.P.	2·5	8750 (1300)	23500 (5600)	6370 (470)	3200 (870)	2070 (300)	1710 (500)	11660 (2900)	6620 (1700)	2150 (370)	3020 (110)	6050 (1900)	3900 (2000)	1880 (120)	2770 (550)	6770 (200)
HSA	10	—	11200 (3470)	4030 (1800)	1280 (80)	1470 (60)	910 (300)	1050 (180)	1560 (60)	1350 (100)	650 (0)	5950 (1500)	2240 (1050)	1410 (130)	1075 (15)	3950 (350)
HSA	25	7270 (1300)	18500 (6100)	4420 (1600)	2050 (950)	1550 (80)	1000 (50)	1054 (300)	2420 (200)	1410 (40)	820 (150)	7530 (3400)	5950 (1520)	1520 (90)	— —	4120 (150)
HSA	37·5	6800 (1100)	—	—	3610 (1500)	1400 (150)	3200 (900)	—	1660 (1000)	1150 (80)	1160 (50)	—	3480 (50)	1610 (50)	2050 (100)	—
URS–HSA	10	6300 (300)	24300 (7100)	7000 (700)	8500 (1600)	2090 (140)	3530 (380)	17260 (500)	6670 (400)	2720 (320)	3300 (1160)	11270 (3500)	4240 (1560)	1770 (240)	2840 (40)	7740 (1300)
URS–HSA	25	6320 (320)	20300 (4700)	7600 (700)	5300 (1340)	1870 (120)	9160 (2000)	12250 (4000)	3215 (380)	4500 (1540)	4090 (6700)	10280 (370)	5320 (1950)	1620 (170)	4900 (550)	4930 (2600)
URS–HSA	37·5	76040 (15100)	15800 (1900)	7550 (500)	10740 (4800)	2020 (400)	8750 (1500)	9260 (3800)	3630 (100)	5480 (700)	4980 (1000)	9900 (4300)	5200 (1260)	1610 (50)	6400 (900)	—
None		5600 (600)	16000 (4200)	3340 (1300)	4210 (640)	1170 (280)	1470 (400)	1160 (230)	1540 (40)	1620 (200)	660 (60)	7260 (2400)	3600 (270)	3040 (1500)	1880 (500)	—
PWM	10λ	—	1300000 (226000)	182000 (1800)	183500 (29200)	113300 (24000)	155400 (8000)	—	119800 (3800)	59400 (4800)	—	157500 (31400)	250000 (1100)	119500 (5400)	—	108600 (27800)

Leukocytes (1 × 10⁶ lymphocytes/ml) were cultured in the presence of various amounts of proteins and urushiol-protein conjugates in Joklik-MEM medium (2 ml) for 5 days. Cells were harvested as described in the text. Incorporation of radioactivity is expressed in disintegrations/min. (DPM). Number in parentheses represent one standard deviation from the mean.

URS, urushiol; S.P., skin proteins; HSA, human serum albumin; PWM, pokeweed mitogen.

Lymphocytes from control donors show a range of stimulation index of 0·6–2·6 in the presence of urushiol–skin protein conjugates and 1·1–3·1 in the case of urushiol–HSA conjugate.

J.C. and C.P. responded to the conjugate of skin proteins and human serum albumin, whereas patients M.L., R.L., F.G. and J.B. responded only to urushiol–albumin conjugate.

TABLE 2. Stimulation index calculated from the ratios of ^{3}H-thymidine incorporation by lymphocytes in culture in the presence of urushiol-protein conjugates over uptake in the presence of carrier proteins alone

		Stimulation index														
		Sensitive donors											Control donors			
Stimulant	Quantity (μg/ml)	M.L.	A.P.	P.B.	R.L.	Y.B.	F.G.	L.L.	J.C.	J.B.	C.P.	D.T.	H.N.	B.R.	R.L.	D.G.
URS–S.P.	1	—	0·8	—	—	1·7	1·2	14·2	5·0	1·4	4·6	1·0	—	—	—	—
URS–S.P.	2·5	1·6	1·4	1·6	2·3	1·5	1·9	11·4	2·8	1·5	4·6	1·0	0·6	0·8	2·6	1·4
URS–HSA	10	—	2·2	1·7	6·6	1·4	3·9	16·4	4·3	2·0	5·1	1·9	1·9	1·3	2·6	2·0
URS–HSA	25	0·9	1·1	1·7	2·6	1·2	9·2	11·7	1·3	3·2	5·0	1·4	0·9	1·1	—	1·2
URS–HSA	37·5	11·2	—	—	3·0	1·4	2·7	—	2·2	4·8	4·3	—	1·5	1·0	3·1	—
S.P./none*	2·5	1·0	1·0	1·2	0·3	1·2	0·6	0·9	1·5	0·9	1·0	0·9	1·7	0·7	0·6	—
HSA/none*	25	1·3	1·1	1·3	0·5	1·3	0·6	0·9	1·6	0·9	1·2	1·0	0·6	0·5	—	—

Stimulation indexes were calculated from results of Table I.

* In these cases, stimulation indexes refer to ^{3}H-thymidine incorporation in the presence of skin proteins or human albumin in comparison to incorporation by cells cultured in medium alone.

URS, urushiol; S.P., skin proteins; HSA, human serum albumin; PWM, pokeweed mitogen.

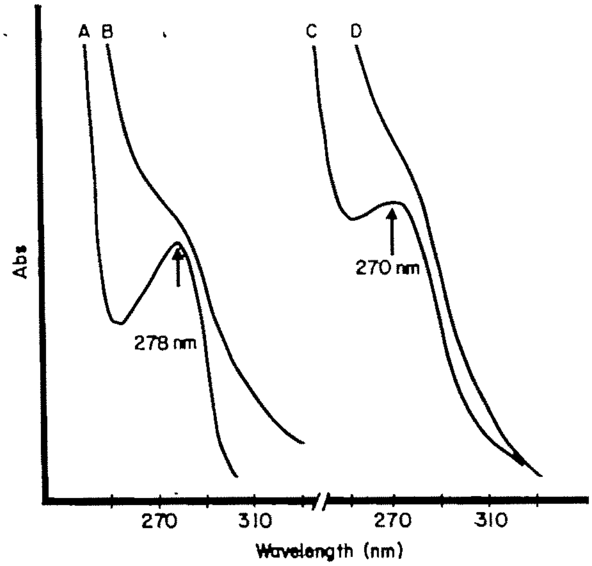

FIGURE 3. Comparative ultraviolet spectra of human serum albumin (A) and its corresponding urushiol conjugate (B), and purified skin proteins (C) and their corresponding urushiol conjugate (D). Spectra were recorded on a Cary 118 spectrophotometer (Varian, Palo Alto, California).

The skin protein extract used in this study did not appear to be antigenic in culture. Except for one case (H.N.), the ratio of radioactivity incorporated by lymphocytes in the presence of skin protein extract (2·5 μg/ml) over incorporation without addition of extract varied from 0·3–1·5. Similarly, when this ratio was calculated for human serum albumin (25 μg/ml), no significant stimulation by HSA was observed (range 0·5–1·6).

The results in Table 2 suggest that patients sensitive to poison ivy may be divided, on the basis of *in vitro* response of their lymphocytes, into two groups: responder and non-responder. In the study reported here, seven donors can be considered to show a positive response to urushiol–protein conjugates *in vitro*, whereas four do not.

DISCUSSION

In the present study, the *in vitro* stimulation of human peripheral lymphocytes by urushiol–protein conjugates has been examined. The protein carriers were selected on the basis of reported studies which have suggested that skin proteins (Milner, 1970, 1971, 1972, 1974; Parker & Turk, 1970; Nishioka *et al.*, 1971; Nakagawa & Tanioku, 1972; Camm *et al.*, 1975) or serum proteins (Hall & Smith, 1971; McFarlin & Balfour, 1973) may serve as hapten carrier in allergic contact dermatitis.

The results (Table 2) show that albumin or the skin protein extract were not stimulants in culture. The ratio of ^{3}H-thymidine incorporation in the presence of skin proteins, or HSA, over incorporation in the absence of these proteins was in the range 0·5–1·7.

Lymphocytes from only three of the sensitive donors responded to urushiol–skin protein conjugate whereas seven responded to urushiol–albumin adduct. These results would suggest that urushiol–human serum albumin is a better antigen than the corresponding skin protein adduct. An alternative explanation would be that, since larger doses of urushiol–HSA were used, the observed results are due to the higher concentration of hapten carrier used. This does not appear to be a reasonable explanation since, in preliminary experiments, it was observed that doses of urushiol–skin protein larger than 2·5 μg/ml were inhibitory. In addition, doses of urushiol–HSA lower than 10 μg/ml were not significantly stimulatory.

The *in vitro* response of human lymphocytes to hapten-carrier conjugates has been reported. Miller & Levis (1973) experimentally sensitized human volunteers, by topical application, to DNCB and used DNP–leukocyte or DNP–erythrocyte conjugates as stimulants in the lymphocyte transformation assay. They observed high stimulation ratios (range 8–25) for DNP-allogeneic leukocytes and for DNP erythrocytes (range 4·0–12·9) when sensitive lymphocytes were used 7–21 days after *in vivo* application of DNCB. A rapid decline in stimulation was observed as early as 3–4 weeks following application of the primary sensitizing dose. Milner (1974) also sensitized human volunteers to DNFB and used DNP–skin protein conjugates in the lymphocyte transformation assay. Stimulation index (50 μg or carrier) range was 2·4–9·3 in the patients showing a strong cutaneous test, and 1·4–5·1 in the less sensitive patients. More recently, Levis, Whalen & Powell (1975) have reported an additional study of human lymphocyte transformation by DNP-erythrocytes (type O). In this study, a stimulation index of 1·1–2·1 was observed in three donors, 4·7–8·5 in three other donors and 22·4–31·0 in the three remaining donors. Cultures were done 20 days after sensitization.

The present study differs from those cited above mainly in the fact that sensitive donors have acquired sensitivity by natural exposure to poison ivy. In addition, except for one case (J.B.), the last recorded exposure was at least 12 months previous to the present study.

The data presented here would suggest that sensitivity to poison ivy can be measured by the lymphocyte transformation assay although as observed here and reported elsewhere (Gimenez-

Camarasa *et al.*, 1975; Milner, 1974), lymphocytes from some hapten-sensitive donors may fail to respond *in vitro*.

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Immune response to human keratoacanthoma

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SUMMARY

Immune reactivity to human keratoacanthoma was investigated by microcytotoxicity tests and immunofluorescence. IgM and complement were consistently present in lesions; IgG and fibrin were infrequent. No evidence of *in vivo* bound immunoglobulin was found on the surface of keratoacanthoma cells by membrane immunofluorescence. Neither patients' sera nor peripheral blood leukocytes showed significant cytotoxicity against autochthonous tumour cells in microtitre assays. This study fails to support the view that regression of human keratoacanthoma is mediated by immunological mechanisms.

Keratoacanthoma generally presents as a rapidly growing lesion which regresses spontaneously, usually within 1 year. The mechanism(s) of regression is unknown, but it is sometimes considered to be of an immune nature. Evidence cited in support of this includes spontaneous regression of lesions, the frequently observed mononuclear infiltrate (Lever, 1967), and the tendency for the infiltrate to increase with the age of the lesion (Calnan & Haber, 1955). The presence of immunoglobulins (IgM, IgG) and complement within the stroma (Brown & Tan, 1973) has also been suggested as evidence of host anti-tumour immunoreactivity. Further evidence of the participation of an immune mechanism is the increased incidence, persistence and recurrence of the lesion in immunosuppressed renal transplant patients (Walder, Robertson & Jeremy, 1971; Jacobsson, Linell & Rausing, 1974; Marshall, 1974; Walder *et al.*, 1976) and in patients with leprosy (Job, 1963) and Hodgkin's disease (Lowry, Clark & Hannemann, 1972), conditions associated with impaired cellular immune responses. In addition, multiple recurrent keratoacanthomas have been reported in a patient with deficient cell-mediated immunity (Claudy & Thivolet, 1975). In this latter case, the lesions regressed following treatment with immunostimulatory drugs.

In spite of this strong circumstantial case for immune control of keratoacanthoma, little evidence has been presented of immunoreactivity directed against the lesion itself. To the authors' knowledge, the positive delayed hypersensitivity skin reactions to autologous keratoacanthoma extracts reported by Nicolau, Badanoiu & Balus (1963) and Maggiora (1968) are the only direct evidence of anti-tumour

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reactivity in the literature. However, Ramselaar & van der Meer (1976) were unable to confirm this. The present paper reports studies on the immunoreactivity of circulating lymphocytes and complement-fixing antibodies against keratoacanthoma, and immunohistological features of the lesion.

MATERIALS AND METHODS

Skin lesions

Thirty-two human skin lesions were excised *in toto* under local anaesthesia; paraffin sections of formalin-fixed tissue were stained with haematoxylin and eosin or methyl green-pyronin (Drury & Wallington, 1967) to demonstrate plasma cells. Diagnosis of keratoacanthoma was based upon clinical history and morphology of the lesions (Rook & Whimster, 1950). The patients (eighteen males, fourteen females) ranged in age from 47 to 90 years (mean 67 years), four of them having a previous history of skin tumours, including one with three previous keratoacanthomas and a squamous cell carcinoma. Lesions were present for 3 weeks to 18 months (mean 18 weeks) and one was known to be regressing when excised.

In addition, blood leukocytes and tumour cells were obtained from two patients with malignant melanoma and from one patient with a cutaneous squamous cell carcinoma, for testing in the leukocyte cytotoxicity assay. In the latter case, serum and tumour cells were also tested in the serum cytotoxicity assay.

Immunofluorescence

For the localization of immunoglobulins, complement and fibrin, nineteen lesions were bisected and one half of each snap-frozen in liquid nitrogen and isopentane and stored at −70°C. Microtome sections (6 μm) were stained by direct immunofluorescence (Nairn, 1976a), using the following fluorescein isothiocyanate-conjugated horse anti-human sera (Netherlands Red Cross): anti-complement (anti-C3 and anti-C4), anti-IgM, anti-IgG, anti-IgA, anti-IgE, anti-IgD and anti-fibrin (F:P ratios 3·9–5·3).

Conjugates were purified by gel filtration through Sephadex G-25, absorbed with rat liver powder and kidney homogenate so that they gave no staining when applied alone to control sections.

In an attempt to detect *in vivo* bound immunoglobulins on tumour cell surfaces, single cell suspensions from six lesions, prepared by gentle teasing with scalpel blades in tissue culture medium 199 (M199, Commonwealth Serum Laboratories, Melbourne), were washed once in medium and stained by direct immunofluorescence with goat anti-human gamma globulin. This had activity against IgG, IgM and IgA, and was absorbed with sheep liver powder, bovine liver homogenate and human group AB erythrocytes.

Microscopic preparations were examined by darkground UV fluorescent microscopy.

Leukocyte cytotoxicity

Autologous leukocytes from fifteen patients were obtained from 10 ml of heparinized blood taken at the time of tumour excision. Control cells were obtained from the blood of normal, healthy donors. Leukocytes were harvested from the buffy coat after standing at 37°C for 1 h, or by separation of whole blood on a discontinuous density gradient of Ficoll-Hypaque (Yamana, Roland & Nairn, 1973). No attempt was made further to separate lymphocytes from other leukocytes. After harvesting, cells were washed once and resuspended in M199 supplemented with 10% fetal calf serum at concentrations of $1{\cdot}25 \times 10^6$ viable cells/ml for use in the microcytotoxicity assay. Viability, assessed by exclusion of 0·1% trypan blue dye, was never less than 90%.

Cytotoxicity assays were carried out in microtitration plates (No. 3034, Falcon Plastics Co.) as described previously (Flannery *et al.*, 1973a). Tumour cell suspensions, prepared in M199 by teasing the lesions gently with scalpel blades, were washed once by centrifugation at 300 *g* max, resuspended, and filtered through a 100 μm mesh copper sieve when necessary. Suspensions of normal epidermal squamous cells were prepared from tissue adjacent to the lesion. Target cells (5×10^4/well) were incubated overnight at 37°C in the microtitration plates and the number of cells adherent to the base of each well after washing was counted by phase contract microscopy. Wells containing 50–100 cells were used in all assays with four to six replicates per well. Leukocyte suspensions (10 μl) were then added to give a final effector to target cell ratio of 200:1 and the plates incubated at 37°C for 40–48 h. Test and control leukocytes were each assayed against tumour and normal skin cells. In some experiments, effector to target cell ratios of 50:1, 100:1 and 1000:1 were also used. After incubation, plates were washed twice, fixed with methanol and the number of tumour cells adhering to each well counted by light or phase contrast microscopy.

Cytotoxicity was expressed as the percentage reduction in the mean number of surviving tumour cells in test (N_t) versus control (N_c) wells, i.e.

$$\% \text{ cytotoxicity} = \frac{N_c - N_t}{N_c} \times 100$$

The statistical significance of cytotoxicity values was assessed by Student's '*t*'-test.

Serum cytotoxicity

Autologous sera were obtained from 10 ml of clotted blood taken from fourteen patients at the time of tumour excision. Fresh control sera were obtained from the blood of fourteen normal, healthy donors. In eight cases, test and control sera were allowed to stand overnight at 4°C before use without the addition of complement. In six cases, test and control sera were heated at 56°C for 30 min to inactivate complement prior to overnight storage at 4°C. Sera were tested for *in vitro* complement-dependent cytotoxicity as previously described (Flannery *et al.*, 1973b). Tumour target cells and normal skin cells were incubated in microtitration plates as described above. After washing and counting, the medium was removed from each well and 5 μl of test or control serum added. Plates incubated with fresh serum (not heat-inactivated) were washed three times after 5 h at 37°C and fixed with methanol. To plates incubated with heat-inactivated serum, 5 μl of a 1:2 dilution of reconstituted, freeze-dried guinea-pig complement (Wellcome Laboratories) was added after 30 min at 37°C and the plates reincubated for a further 5 h, after which they were washed and fixed. Cytotoxicity was determined as described above.

RESULTS

Histopathology

The gross morphology and light microscopic appearance of keratoacanthoma have been described previously (Lever, 1967; Muller & Flannery, 1973). Mononuclear infiltration of the base of the lesions was a common finding but of variable intensity. The infiltrate, consisting of lymphocytes, plasma cells and eosinophils, was marked in the regressing lesion (Fig. 1), but intense infiltrates were also observed in several actively growing lesions.

Immunohistology

In nineteen lesions, direct immunofluorescence showed bright IgM (Fig. 2) and complement (C3 and C4) cytoid staining in the dermis around epidermal projections. The distribution of the IgM staining corresponded in part to the presence of plasma cells as demonstrated by methyl green-pyronin. In only three cases was weak cytoid staining detected with anti-IgG. All lesions failed to stain with anti-IgA, anti-IgE or anti-IgD. In five lesions fibrin was present in the tumour stroma.

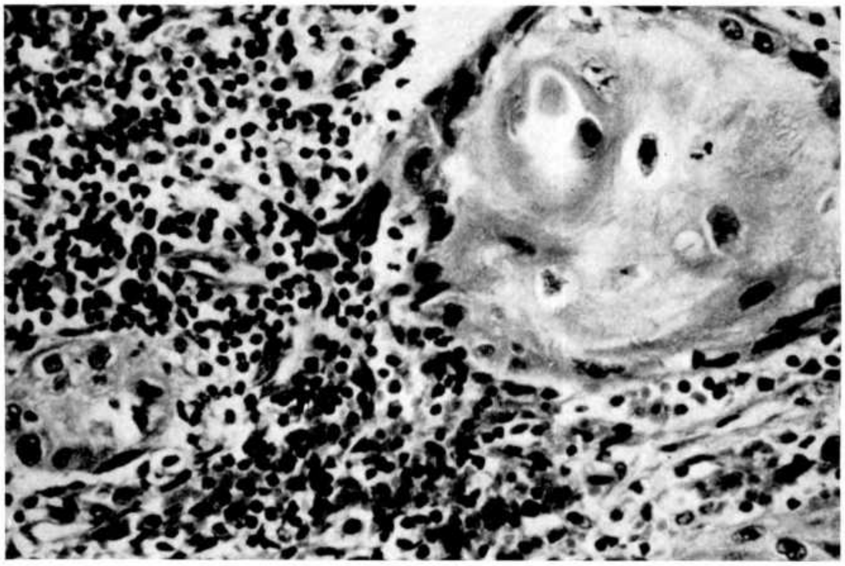

FIGURE 1. Advanced keratinization in a regressing keratoacanthoma. Prominent mononuclear infiltrate surrounds epidermal projections (H & E, × 420).

No staining was observed in the intercellular area or around individual cells in the epidermal projections.

No evidence of *in vivo* bound immunoglobulin was found on the surface of tumour cells in suspensions stained with anti-immunoglobulin serum.

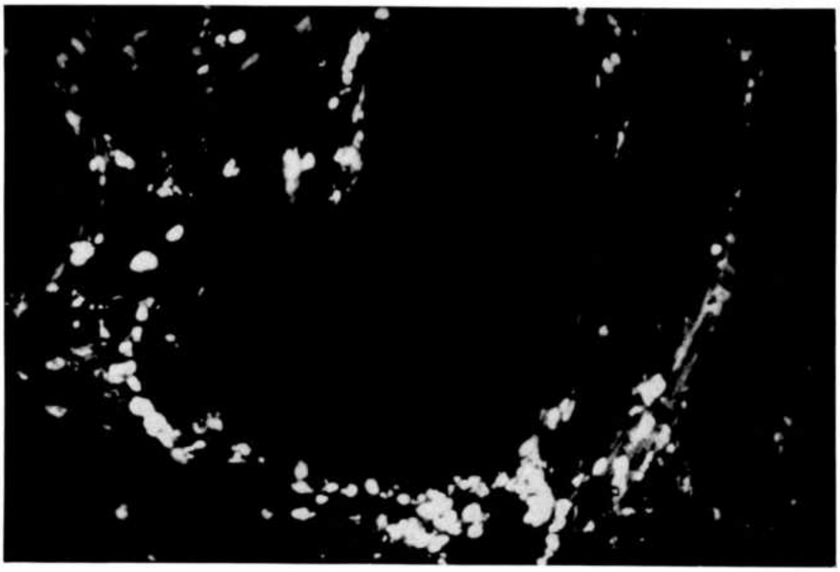

FIGURE 2. IgM cytoid staining around epidermal projection in growing keratoacanthoma (direct immunofluorescence, × 200).

Leukocyte cytotoxicity

Throughout the microcytotoxicity assays, tumour and normal skin cells appeared viable, as judged by light and phase contrast microscopy, but showed no evidence of proliferation. Keratoacanthoma cells adhered to plastic and generally had the appearance of normal squamous cells although occasionally they tended to overgrow each other as tumour cells do (Fig. 3). Patients' and normal control mononuclear cells were sometimes attached to tumour cells in culture (Fig. 3).

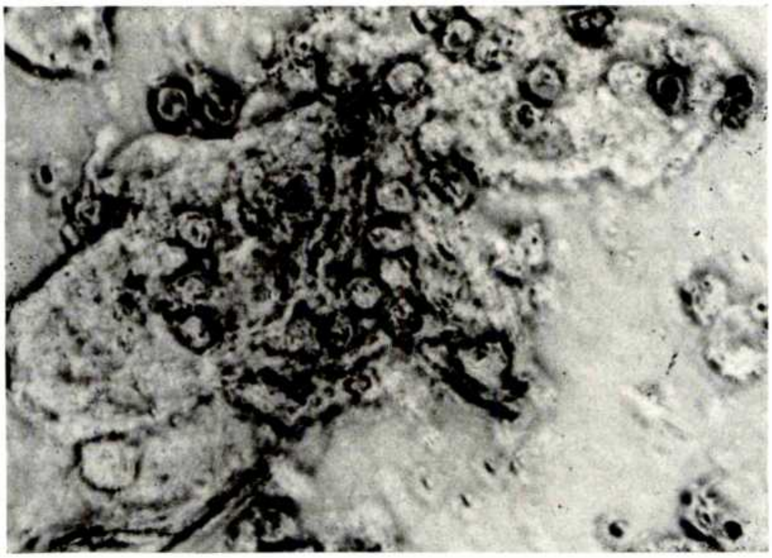

FIGURE 3. Keratoacanthoma cells after 40 h growth in microtitration plate. Mononuclear cells adhere to overgrowing squamous cells (phase contrast, × 800).

No significant cytotoxicity against autologous tumour target cells was found using peripheral blood leukocytes, even at effector to target cell ratios as high as 1000:1. Cytotoxicity values ranged from 0 to 24% (mean 8%) and although the highest value was obtained with leukocytes from the patient whose keratoacanthoma was known to be regressing at the time of excision, no value was statistically significant.

In contrast, significant cytotoxicity was observed in both cases of malignant melanoma (25% and 28% respectively, $P<0{\cdot}05$) and in the case of squamous cell carcinoma (32%, $P<0{\cdot}05$).

Serum cytotoxicity

Neither fresh sera nor heat-inactivated sera with added complement were found to be cytotoxic to autologous tumour or normal skin cells in fourteen cases of keratoacanthoma (cytotoxicity range 0–17%, mean 5%) and the single case of squamous cell carcinoma.

DISCUSSION

The results of the present study fail to support the view that spontaneous regression of human keratoacanthoma is mediated by an immunological mechanism. In no case were blood leukocytes from patients with keratoacanthoma significantly reactive against autologous target cells *in vitro*, while blood leukocytes were reactive against autologous malignant melanoma and squamous cell carcinoma.

These tests suggest that specifically cytotoxic T lymphocytes are either absent from the peripheral blood, or that they are inhibited by serum factors as are reported in many tumour systems (Hellström & Hellström, 1974). The absence of circulating cytotoxic cells might be due to trapping within the tumour, or to the failure of this localized lesion to evoke a T-cell response. Since no serum was added in the leukocyte assay, serum blocking factors, if present, must be bound to the effector cells or target cells *in vivo*. We have no evidence that this is the case, and immunofluorescence failed to detect *in vivo* bound immunoglobulin on tumour cells.

Antibody-dependent cell-mediated cytotoxicity cannot be excluded on the basis of these results since the low antibody levels required to activate K-cell killing are beyond the sensitivity of the immunofluorescence technique (Möller & Svehag, 1972). The addition of normal leukocytes and patients' sera at high dilution in the microcytotoxicity assay should resolve this question.

No cytotoxic reactivity directed against keratoacanthoma cells was observed using either fresh autologous sera or heat inactivated sera with added complement. Thus it seems unlikely that complement-dependent lysis mediated by antibody plays a significant part in the regression of the lesion. This is in contrast to the higher frequency of cytotoxic antibodies found in the sera of patients with malignant melanoma (Nairn, 1974) and squamous cell carcinoma of skin (Nairn, 1976b) and might reflect the relatively low immunogenicity of the keratoacanthoma.

In view of the above, the significance of the positive IgM and complement cytoid staining around the epidermal projections is unclear. In part, the IgM staining was associated with plasma cells. A viral aetiology has been suggested for keratoacanthoma (Prieto *et al.*, 1964) although attempts to demonstrate virus in human lesions have to date been unsuccessful (Fisher, McCoy & Wechsler, 1972). It is possible, then, that the localization of IgM in our study and that of Brown & Tan (1973) represents host response to virus or some other antigen and not anti-tumour reactivity.

In contrast to the study of Brown & Tan (1973), IgG and fibrin were infrequently observed in the present investigation. In particular, they detected IgG in areas of cell infiltration and microabscesses accompanied by IgM and C3. While these observations might reflect an immune reaction, it is also possible that they are the result of secondary inflammatory changes associated with advanced lesions showing cellular degeneration and necrosis.

The age of lesions at the time of presentation is difficult to determine (Fisher, 1969). The lesions in our study had been present for at least 3 weeks (mean 18 weeks), but it is possible that several keratoacanthomas had not reached maturity, and only one was known to be regressing at the time of testing. In view of the leukocyte cytotoxicity result from the latter patient, it may be that only in regression is immunoreactivity detectable, again in contrast to the findings with growing skin tumours of other histological types (Nairn, 1974, 1976b).

The failure to demonstrate immunological reactivity against keratoacanthoma raises the possibility of non-immune regression. Andrews (1971) has reported regression of transplanted papillomas in immunosuppressed animals, also suggesting a non-immune mechanism. No such experiments have as yet been reported for experimental keratoacanthoma. Prutkin & Gerstner (1966) have suggested that mechanical factors, such as 'shedding of brittle, keratinized epithelium', might be important in the regression of keratoacanthoma, adding however that 'other factors must exist', and Ghadially (1958) has stressed the effect of the inflammatory infiltrate.

In the case of human viral warts, where immune responses are well documented (reviewed by Lee & Eisinger, 1976), the limited life span of transformed cells, and hence regression, may be associated with spontaneous cessation of virus production (Pyrhönen & Johansson, 1975). A similar mechanism might be envisaged for keratoacanthoma with cessation of the initiating agent.

Our findings then, fail to provide direct evidence that regression of human keratoacanthoma is mediated by immune mechanisms.

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Complement C3 and immunoglobulin in inflammatory acne vulgaris

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SUMMARY

In patients with moderate to severe inflammatory acne, complement (C3) was detected by immunofluorescence in sixteen of sixteen early inflammatory acne lesions but in only one of thirteen biopsies of non-inflamed skin from acne sites. C3 deposition occurred particularly in the walls of small dermal blood vessels and at the dermo–epidermal junction. IgM was identified in vessel walls in four of sixteen early lesions. In eight late inflammatory lesions C3 deposition was much less prominent and was present in vessel walls in only two. None of the late lesions showed vascular deposition of IgM.

The observations indicate that complement activation occurs in inflammatory acne and it is suggested that this may play a pathogenic role in the inflammation.

Despite extensive investigation, the mechanism of inflammation in acne is unclear. Considerable evidence has suggested a pathogenic role for *Propionibacterium* (*Corynebacterium*) *acnes*. It has been suggested (Strauss & Pochi, 1965) that inflammation is induced by irritant fatty acids derived from sebum by bacterial lipase activity. However, the evidence in favour of this does not stand up to critical review (Voss, 1974) or more recent experimental evidence (Puhvel & Sakamoto, 1977). Complement fixing antibodies to *P. acnes* have been found in patients with severe inflammatory acne (Puhvel, Hoffman & Sternberg, 1966) and we have previously reported briefly on the observation of complement C3 deposition in early inflammatory acne lesions (Dahl & McGibbon, 1976). In this paper we report the results of a more detailed study.

PATIENTS AND METHODS

Thirty-seven skin biopsies were taken from twenty patients with moderate to severe inflammatory acne. All except one were male and their ages ranged from 15 to 32 years (mean 20). Biopsies were taken from the back, chest or upper arms and comprised sixteen early inflammatory lesions, eight

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late inflammatory lesions and thirteen specimens of non-inflamed skin from acne areas. The precise age of the individual lesions was not known but early lesions were selected on the basis of the patient's history and the clinical signs of bright erythema, tenderness, induration and the absence of (or only slight) pus formation. Early lesions were approximately 0·5–1·0 cm in diameter and it was estimated that they had been present for 2–7 days. Old lesions had been present for 2–8 weeks according to the patients.

Biopsy specimens were obtained using local anaesthesia with lignocaine. Specimens were immediately embedded in phosphate buffered gelatin, frozen in liquid nitrogen and stored at −30°C for up to 2 weeks before preparation of 5 μm cryostat sections. Sections were washed in two changes of phosphate buffered saline for 15 min on each occasion and processed by methods slightly modified from those of Lai A Fat, Cormane & van Furth (1974). Goat anti-human sera conjugated with fluorescein isothiocyanate and specific for IgG, IgM, IgA and C3 were obtained from Nordic Immunological Laboratories Ltd. A goat anti-human C3 serum from Meloy Laboratories was also used. The specifications and details of the antisera used are shown in Table 1. Sections were viewed at a magnification of ×240 in a Zeiss (W. Germany) fluorescence microscope fitted with an XBO 150 Xenon lamp source, blue narrow-band excitation filters and incident illumination.

TABLE 1. Details of fluorescein-conjugated antisera

Type	Protein content (mg/mm^3)	Fluorescein/ protein ratio (M)	Dilution
Goat anti-human IgG/FITC Nordic, batch no. 2.372	8·9	1·1	1/30
Goat anti-human IgM/FITC Nordic, batch no. 15.173	14·2	1·2	1/20
Goat anti-human IgA/FITC Nordic, batch no. 1172	12·2	1·3	1/30
Goat anti-human C3/FITC Nordic, batch no. 27.575	9·6	1·9	1/30
Goat anti-human C3/FITC Meloy, batch no. 64394	11·3	2·7	1/40

RESULTS

The findings in early inflammatory lesions are summarized in Table 2. The most prominent and constant feature was the deposition of C3 in the walls of small dermal blood vessels in fourteen of sixteen specimens. Identical results were obtained with the two different C3 antisera. In several biopsies ten to twenty involved vessels could be found in a single section. At the periphery of the inflammatory lesions the vessel walls appeared relatively undamaged and C3 was located particularly on the luminal aspect of endothelial cells (Fig. 1). Towards the central areas of inflammation heavier and more diffuse C3 deposition was found in the vessel walls together with perivascular granular deposits (Fig. 2). In the vicinity of microscopic abscess formation there was often gross disruption of vessel walls (Fig. 3). C3 deposition was noted in papillary vessels in five of sixteen early inflammatory lesions accompanied by granular deposition along the dermo-epidermal junction (Fig. 4). A further eight biopsies showed the granular junctional deposits without involvement of papillary vessels.

TABLE 2. Deposition of C3 and immunoglobulin in early inflammatory acne lesions

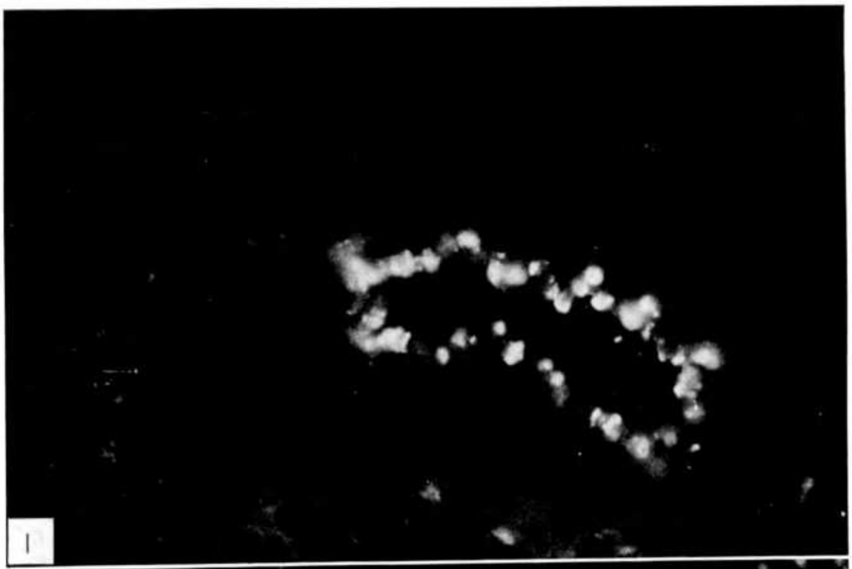
	Complement C3			Immunoglobulin and C3 in dermis		
Patient No.	Dermal vessels	Papillary vessels	Dermo–epidermal junction	Dermal vessels	Extravascular granular aggregates	Cells
1	+	+	+	M	G,M,C3	—
2	—	—	+	—	M,A	M
3	+	+	+	—	M,A	M,A
4	+	—	—	—	M,A,C3	—
5	+	+	+	M	M,A	—
6	+	—	+	—	G,M,A,C3	—
7	+	—	+	—	G,C3	A
8	+	—	+	M	M,A	M.A
9	+	—	+	M	M,A,C3	M,A
10	+	—	—	—	C3	—
10	—	+	+	—	G,M,A	—
12	+	—	+	—	—	—
13	+	+	+	—	—	—
14	+	—	—	—	—	—
15	+	—	—	—	—	—
16	+	—	+	—	M,A	—

IgM was detectable in the walls of dermal blood vessels in four of sixteen early inflammatory lesions. The staining pattern was comparatively weak (Fig. 5) and in serial sections it was not possible to show both IgM and C3 deposition in the same vessels in any of the specimens. No IgG or IgA was found in vessel walls.

In the late inflammatory lesions (Table 3) C3 deposition in dermal vessels was present in only two of eight biopsies. C3 deposition at the dermo–epidermal junction was observed in six but in three the pattern was diffuse and homogeneous. No involvement of papillary vessels was found.

In the thirteen biopsies of uninflamed skin (Patients 7–12 and 14–20) C3 was found in only one (Patient 8). In this biopsy a single vessel was found with small amounts of C3 mainly localized to the luminal aspect of the endothelium (Fig. 6).

Granular extravascular and extracellular deposits of IgG, IgM, IgA and C3 were found in the dermis and were particularly common and conspicuous in early inflammatory lesions (Table 2) of which twelve of sixteen were positive. The immunoglobulin class was not consistent and similar, though much sparser aggregates were present in three of eight late lesions (Table 3) and two of thirteen uninvolved biopsies. One of the latter (Patient 8) showed granular deposits of IgM and IgA and was the single non-inflammatory specimen in which C3 had been found in a vessel wall. Cells showing fluorescence for IgM and for IgA were found in five of sixteen early lesions, three of eight late lesions (including IgG in one) and four of thirteen uninvolved biopsies. The nature of these cells has not been determined.



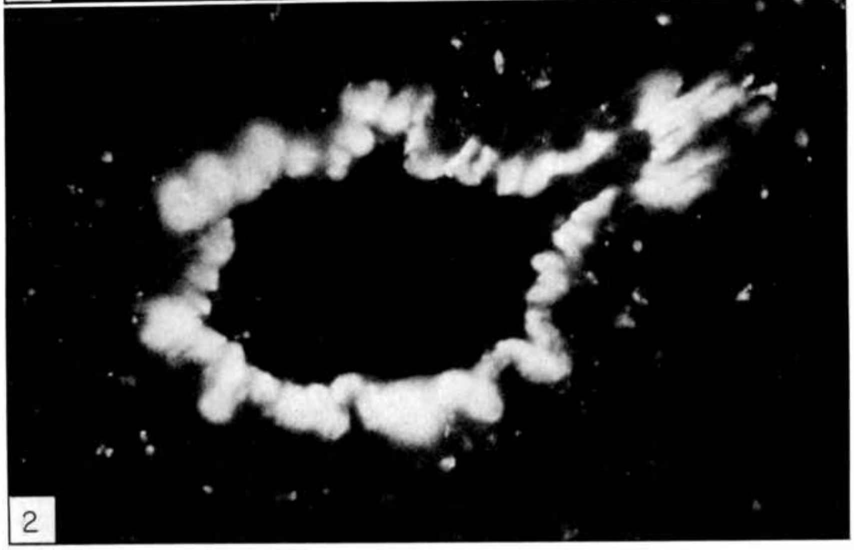
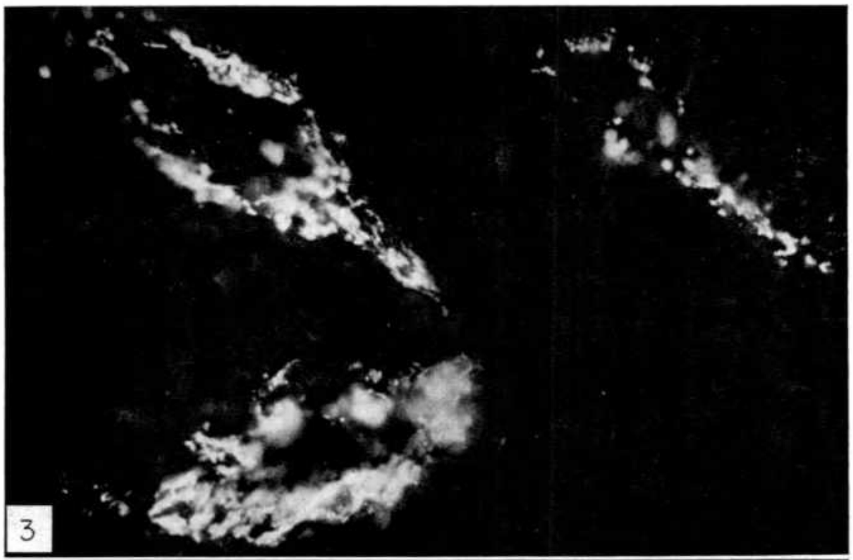


FIGURE 1. Complement C3 in mid-dermal blood vessel from the edge of an early inflammatory acne lesion. The fluorescence is maximal on the luminal aspect of endothelial cells and the vessel wall appears largely undamaged.

FIGURE 2. Complement C3 in a mid-dermal vessel from early inflammatory acne. Compared with Fig. 1 the deposits appear heavier and there is additional granular perivascular deposition.



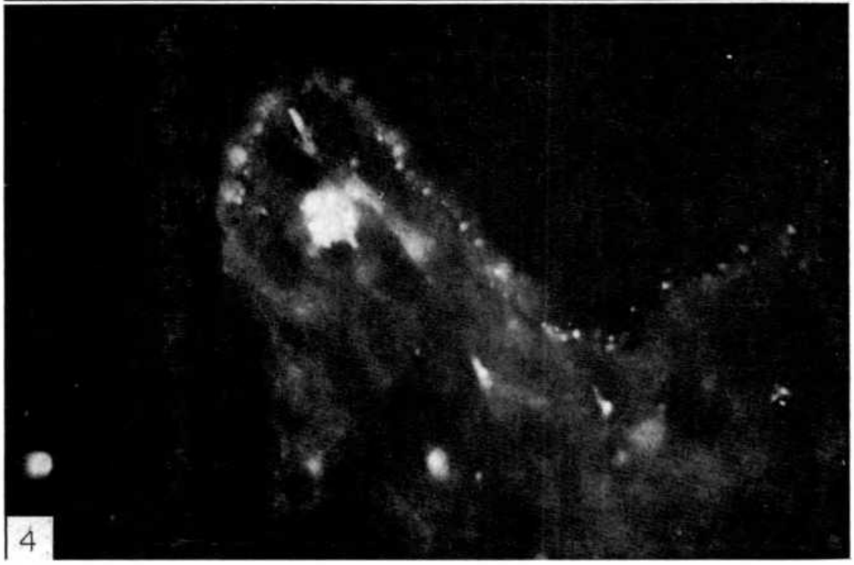
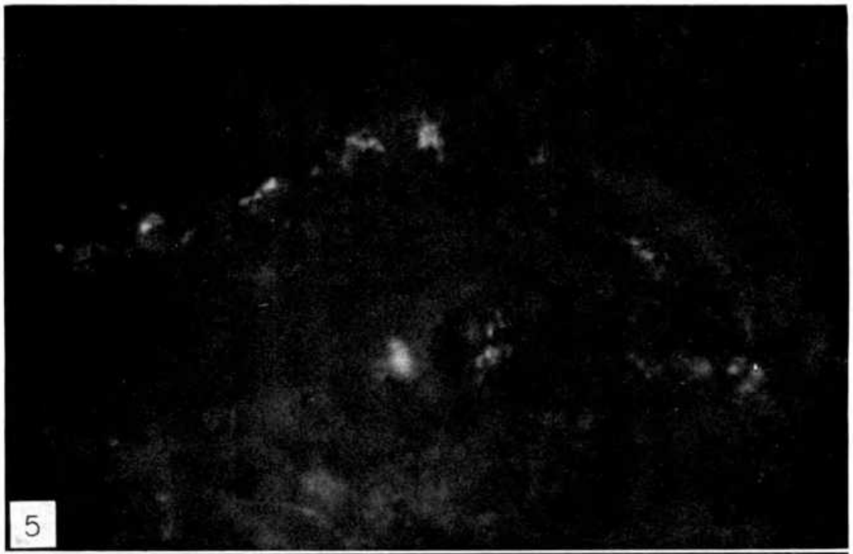


FIGURE 3. Gross disruption of dermal vessels showing fluorescence for C3. This appearance was found around areas of microscopic abscess formation.

FIGURE 4. Complement C3 in a papillary vessel with granular C3 deposition along the dermo-epidermal junction.



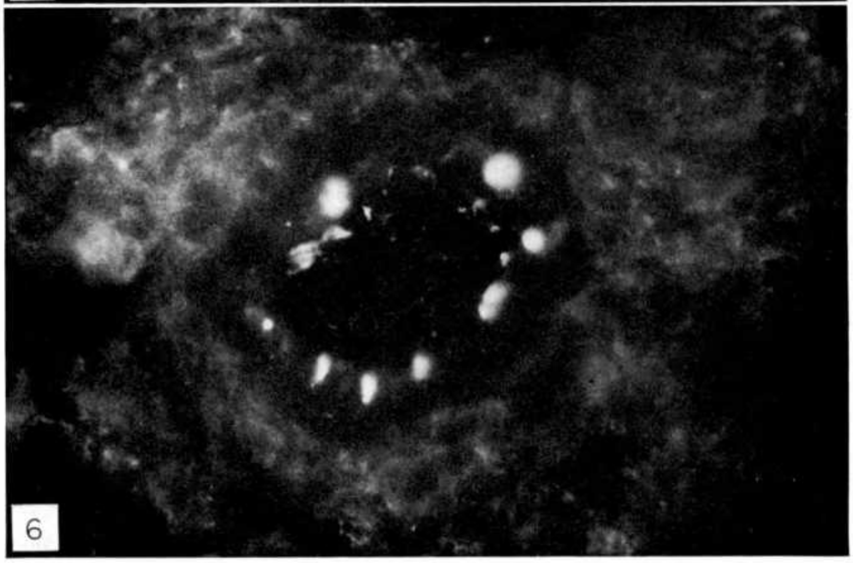


FIGURE 5. IgM in dermal vessel walls of an early inflammatory acne lesion. One vessel is sectioned transversely (lower right centre) while another (above) is cut longitudinally.

FIGURE 6. Uninvolved skin (Patient 8). Localized areas of C3 deposition are present in the endothelium. This was the only involved vessel found in the thirteen control biopsies from non-inflamed skin.

TABLE 3. Deposition of C3 and immunoglobulin in late inflammatory acne lesions

	Complement C3			Immunoglobulin		
Patient No.	Dermal vessels	Papillary vessels	Dermo-epidermal junction	Dermal vessels	Extravascular granular aggregates	Cells
13	—	—	+	—	—	M,A
14	—	—	+*	—	—	—
15	—	—	—	—	—	—
16	—	—	+	—	M	—
17	—	—	+	—	M,C3	M
18	—	—	+*	—	—	—
19	+	—	—	—	—	—
20	+	—	+	—	G,M,A	G,M,A

* Diffuse homogeneous pattern.

DISCUSSION

The most conspicuous observation in this study was the presence of C3 in the walls of dermal vessels. With one exception, this feature was confined to lesional skin and was found most frequently in early inflammatory lesions. It may be that the single vessel staining for C3 in one of thirteen uninvolved specimens represented a preclinical or subclinical lesion. Activation of the complement system leads to the release of inflammatory mediators and chemotactic factors (reviewed by Gigli, 1976). The association of C3 with inflammatory lesions therefore suggests that complement activation may play a pathogenic role in the inflammation of acne.

Scott, Cunliffe & Gowland (1979) have recently described similar deposition of C3 and occasionally C1q in dermal vessels of inflammatory acne lesions. However they also made similar observations in relation to non-inflammatory comedones. In the present investigation comedones were not specifically studied, but we observed vascular C3 deposition in only one of thirteen biopsies of non-inflamed skin from acne areas. Resolution of this apparent discrepancy is important in considering the significance of complement activation in the initiation of inflammation. Scott *et al.* (1979) also reported the deposition of C3 in the basement membrane zone of the involved pilosebaceous unit in both inflammatory acne lesions and comedones. Although we have made similar observations (unpublished) in biopsies of acne subjects we have also found an identical pattern of C3 staining in non-lesional biopsies from various sites including the scalp and thighs in patients with a variety of other skin conditions, e.g. alopecia areata and cutaneous vasculitis. The pilosebaceous basement membrane pattern of C3 deposition therefore seems to be specific neither for acne nor for acne sites.

Complement fixing antibodies to *P. acnes* have been found in patients with inflammatory acne (Puhvel *et al.*, 1966). The demonstration of immunoglobulin as well as C3 raises the possibility that complement activation may occur via the immune classical pathway. Immunoglobulin was present in both early and late lesions as well as in some uninvolved biopsy specimens, but was found much more conspicuously and frequently in early lesions. Deposition of IgM in dermal vessel walls was confined to early lesions. Nevertheless in none of our biopsies was it possible in serial sections to demonstrate immunoglobulin and C3 at the same sites. It is therefore by no means clear that the C3 deposits which we have observed result directly from an immune reaction initiated by the complexing of immunoglobulin with antigen. Activation of the alternate complement pathway by *P. acnes* has been demonstrated *in vitro* (McBride *et al.*, 1975; Massey, Mowbray & Noble, 1978) and this and the C3b amplification system (Gigli, 1976) are possible alternative mechanisms which may account for our observations. Since C3 is common to all these complement activation pathways our observations do not distinguish between them.

Extravascular immunoglobulin and C3 were very variable in amount and distribution. Interpretation of their significance can be only speculative. Increased vascular permeability associated with the inflammation is likely to allow increasing amounts of antibody to leak into extravascular tissues. Under these circumstances, extravascular complexing of antibody with *P. acnes* and perhaps other antigens may occur. The extracellular deposits of immunoglobulin and C3 may therefore represent locally formed immune complexes while the intracellular deposits could be either immune complexes engulfed by polymorphs or local immunoglobulin synthesis by plasma cells. These possibilities could be resolved only by more specialized immuno-histochemical techniques.

In addition to the demonstration of complement fixing antibodies to *P. acnes*, other immune reactions related to *P. acnes* including lymphocyte transformation (Puhvel & Sakamoto, 1977) and macrophage migration inhibition (Gowland *et al.*, 1978) have now been described in patients with acne. Although the present observations do not directly implicate *P. acnes* they do support the idea of a role for immunological mechanisms in inflammatory acne.

ACKNOWLEDGMENTS

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Effect of dicarboxylic acids on normal human melanocytes in dispersed tissue culture*

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SUMMARY

Since dicarboxylic acids are competitive inhibitors of tyrosinase, and effective in treatment of hyperpigmentary disorders, such as chloasma and lentigo maligna, probably due to a cytotoxic effect on abnormal melanocytes, it is of interest to examine their effect on normal melanocytes in tissue culture. Azelaic or dodecandioic acids were added (150–200 μg/ml) to dispersed cultures of epidermal cells, and melanocytes were examined by electron microscopy after 7, 10, 15, 20 and 30 days. Apart from a stimulation of melanogenesis, the presence of dicarboxylic acids in the culture medium caused no detectable damage to melanocytes, nor did they prevent growth of a second generation of cells.

Localized epidermal depigmentation, frequently preceded by transient hyperpigmentation, is a feature of pityriasis versicolor, and is accompanied by degenerative changes of varying degrees in many melanocytes within the areas of fungal infestation (Breathnach *et al.*, 1975; Grupper, Cesarini & Prunieras, 1975). There is evidence (Nazzaro-Porro & Passi, 1977) that these effects may be caused by products formed through the oxidation by a *Pityrosporum* enzyme system of the double bond linkages of unsaturated fatty acids normally present in skin surface lipids, and that their mode of action involves inhibition of the enzyme tyrosinase. Recently (Nazzaro-Porro & Passi, 1978a), it has been established from cultures of *Pityrosporum* supplemented with oleic or vaccenic acid that the products in question are dicarboxylic acids, notably azelaic (C_9) acid and dodecandioic (C_{12}) acid, and that they are competitive inhibitors of, and not alternative substrates for, tyrosinase; it has also been shown that commercial pure standard dicarboxylic acids from C_8 to C_{14} are likewise capable of inhibiting tyrosinase activity *in vitro*. Observations on hyperpigmented disorders of human skin (Nazzaro-Porro & Passi, 1978b; Nazzaro-Porro *et al.*, 1979) and on transplanted Harding-Passey melanoma in mice

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* A preliminary report of this study was presented at the Annual Meeting of the British Association of Dermatologists, Nottingham, July 1978.

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(Nazzaro-Porro *et al.*, 1978) further indicate that dicarboxylic acids may have a direct cytotoxic action on hyperactive and structurally abnormal melanocytes, possibly related to or stemming from tyrosinase inhibition. As an initial step towards investigating this matter, their effect on normal human melanocytes in tissue culture has been studied.

MATERIALS AND METHODS

Human skin of different degrees of pigmentation was obtained at plastic surgery or circumcision. Pieces of whole skin were stretched and pinned around a sterilized cork and thin slices were cut freehand, using a razor blade held in Spencer Wells forceps. The slices, consisting mostly of epidermis, with a supporting framework of dermis, were floated, dermis side down, on a solution of 0·25% trypsin in Dulbecco's saline. This was incubated at 37°C for at least 20 min, until the epidermis could be lifted off the dermis. The epidermis was then transferred to the culture medium, and shaken, to release the loosened cells. The culture medium was Eagles MEM with HEPES salts and glutamine, and 18% fetal calf serum, and cultures were incubated at 37°C in Falcon tissue culture flasks (Biocult Ltd) each containing 5 ml of medium.

Both azelaic acid and dodecandioic acid were dissolved in ethanol at a concentration of 5 mg/ml, and the ethanolic solution was added to the culture medium to give final concentrations of 150 μg/ml, and 200 μg/ml, for experimental cultures. An equivalent volume of ethanol was added to culture medium for control cultures, and both types of culture were grown for periods up to 30 days. Fresh dicarboxylic acid was added to cultures at intervals of 4 days.

After 7, 10, 15, 20, and 30 days, culture medium was decanted from flasks, and the cells briefly rinsed with Dulbecco to remove surplus serum. This was then replaced with 3% glutaraldehyde in 0·1 M cacodylate buffer at pH 7·4 for 1 h, and after washing in buffer the cells were post-fixed in 2% aqueous osmium tetroxide for 1 h. The cells were then dehydrated in graded ethanols, and at the 70% stage, were scraped off the plastic with a teflon coated spatula, and spun down to a pellet. After complete dehydration, the pellet was embedded in Spurr resin, and thin sections, stained on the grid with uranyl acetate and lead citrate, were examined by electron microscopy.

RESULTS

Seven and 10 *day cultures*

At 7 and 10 days, two morphological types of melanocytes were present in both control and experimental cultures: (a) rounded cells with occasional narrow dendrites; and (b) elongated, spindle-shaped cells. With both types of cell, though more evidently with the former, the number of melanosomes and their degree of melanization were significantly greater in the experimental as compared with the control cultures (Fig. 1); these effects were more noticeable in those exposed to dodecandioic acid than to azelaic acid. These differences, though present in cultures derived from skin of varying degrees of original pigmentation, were more evident with melanocytes of the more lightly pigmented specimens. In all cultures the overall ultrastructural appearance of melanocytes in terms of general cytoplasmic organelles and membranes was good, and with the experimental ones nothing was observed in this connection which could be attributed to a deleterious effect of the addition of dicarboxylic acids to the medium (Fig. 2). The general appearance of keratocytes also seemed to be unaffected

With melanocytes in all circumstances the majority of melanosomes were spheroidal in shape, with predominantly granular rather than laminar internal structure, and many of the Stage 1 and Stage 2 granules present in the spindle-shaped cells exhibited irregular melanization (Fig. 2). These are features usually associated with 'phaeomelanosomes', and they were characteristic even of melano-

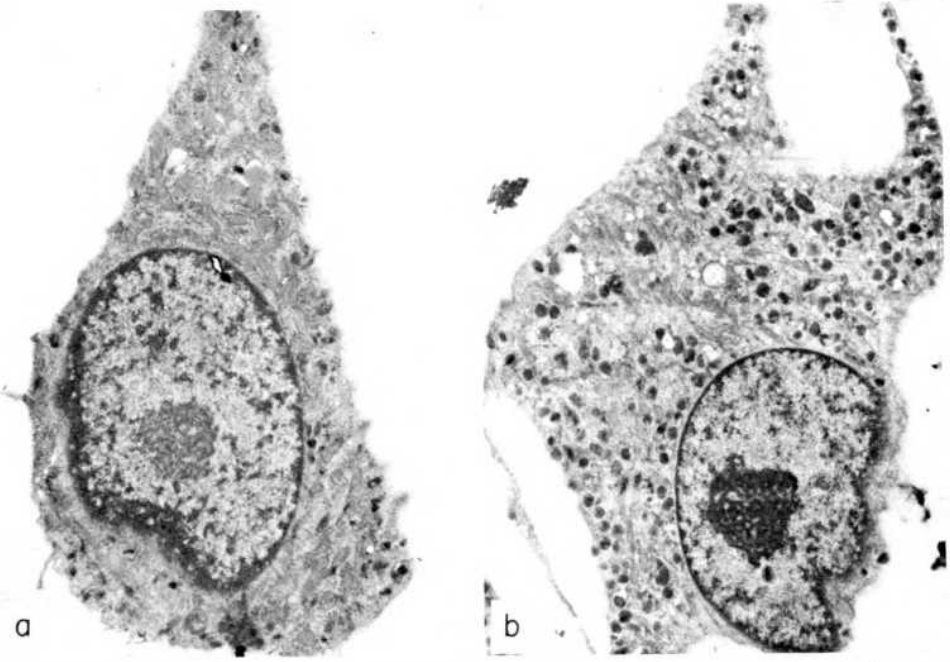

FIGURE 1. (a) Melanocyte from 7-day control culture (× 6000). (b) Melanocyte from culture to which dodecandioic acid was added. Note that in comparison to control (a) there are more numerous and more heavily melanized melanosomes. The general appearance of the cell is however quite healthy (× 6000).

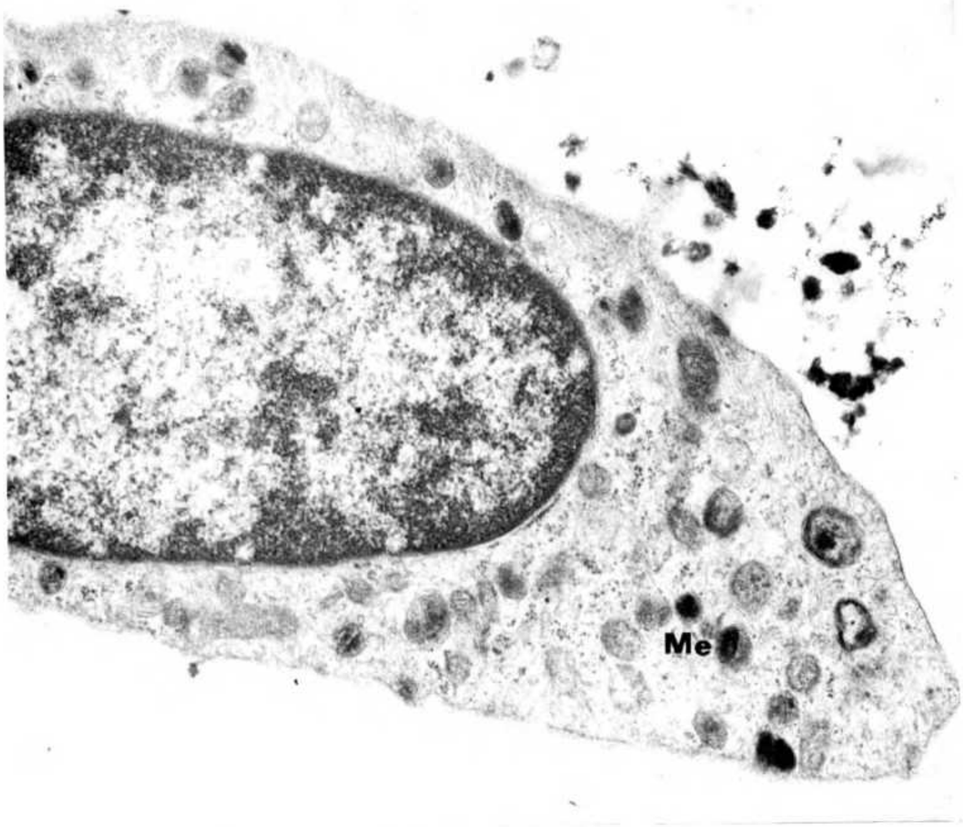

FIGURE 2. Spindle-shaped cell from 10-day dodecandioic acid culture. Nucleus and cytoplasm appear quite normal and healthy. Melanosomes (me) are mainly of spheroidal type, and exhibit irregular melanization (× 22,500).

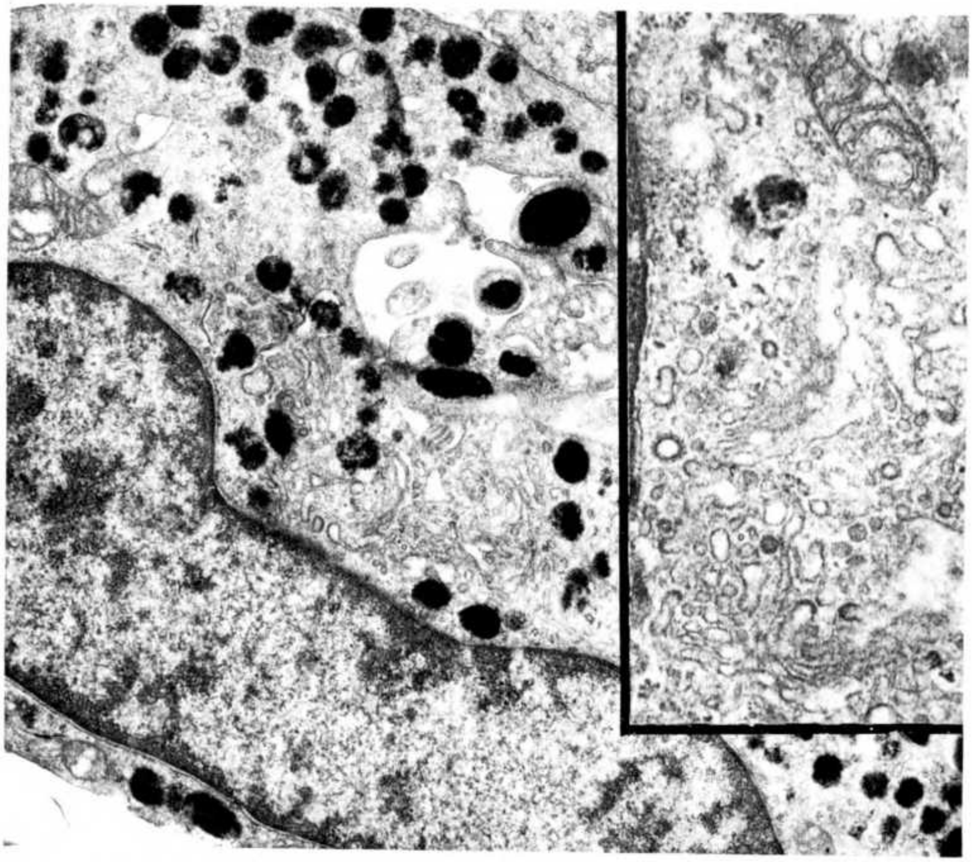

FIGURE 3. Melanocyte from 15-day dodecandioic acid culture. Melanosomes are heavily melanized and were much more numerous than in corresponding 15-day control melanocytes. The general appearance of this cell is quite healthy (× 18,000). Inset: Golgi region of melanocyte from 15-day azelaic acid culture; mitochondria, Golgi membranes and vesicles appear normal and unaffected by exposure to the dicarboxylic acid (× 46,000).

somes of cells cultured from heavily pigmented skin, which evidently had been producing ellipsoidal 'eumelanosomes' previously.

Fifteen day cultures

Melanocytes exhibiting a variety of features were present at this stage in all cultures, both control and experimental. These were: (1) rounded, and spindle-shaped cells of healthy general appearance (Fig. 3), as in the 7-and-10 day cultures, and, as with these latter, the number and degree of melanization of melanosomes was significantly greater in the experimental as compared with the control cultures; (2) cells containing many spheroidal organelles exhibiting irregular electron density, the majority of which appeared to be lysosomal in nature, but some of which could have been enlarged spheroidal melanosomes exhibiting irregular melanization (Fig. 4); with many individual organelles it was often difficult to decide which; (3) cells with cytoplasm riddled with round vacuoles, either empty, or containing melanosomes or remnants thereof (Fig. 5); appearances suggested that these vacuoles were derived both from single melanosomes which had become swollen as well as from compound melanosomes, or phagosomes.

Taking into account the different appearances exhibited by the variety of melanocytes present at this stage in all cultures, the only feature that could in any way be attributed to the addition of dicarboxylic acids to the culture medium was, as at 7 and 10 days, an apparent stimulation of melanogenesis; otherwise, there were no significant differences between control and experimental cultures.

Twenty and 30 day cultures

At 20 days, melanocytes of all cultures were predominantly spindle-shaped (Fig. 6), and vacuolated cells so characteristic of 15-day cultures and presumably undergoing degeneration, were very rarely seen. The general ultrastructural appearance of the cells was good, and in this connection, again, no difference was observed between melanocytes of control and experimental cultures. The majority of melanosomes within the cells were at Stage 1 or early Stage 2, mainly round in shape, and in general their number and degree of melanization seemed to be marginally greater with the experimental cultures, and somewhat more so with cells exposed to dodecandioic acid than to azelaic acid (Fig. 6).

At 30 days, all cultures exhibited a variety of cell types similar to that observed at 10–15 days,

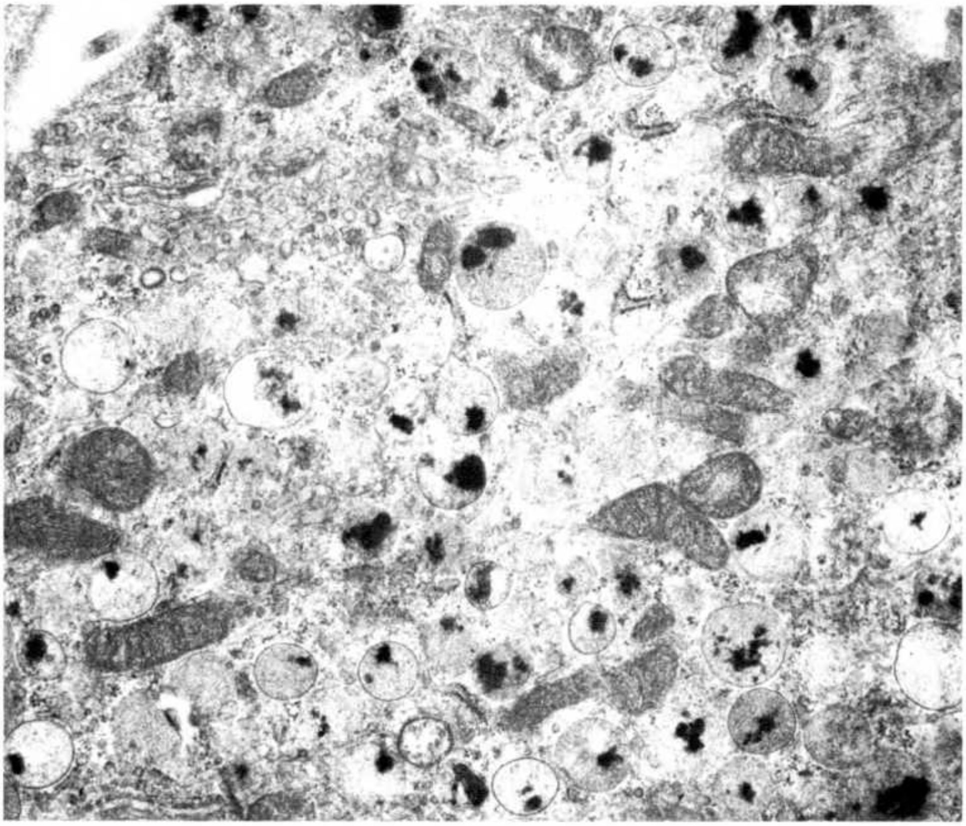

FIGURE 4. Melanocyte from 15-day control culture. Membrane-limited spheroidal organelles of various size with electron-dense content are present in the cytoplasm. The majority of these are probably lysosomal in nature, but some could be melanosomes. In any case, this melanocyte is obviously less heavily melanized than that in Fig. 3 (× 23,500).

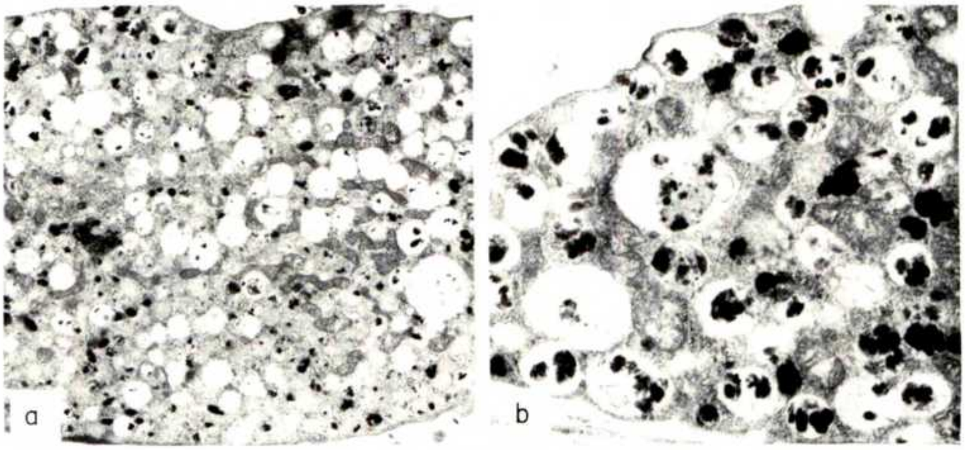

FIGURE 5. (a) Melanocyte from 15-day control culture exhibiting numerous cytoplasmic vacuoles, many containing melanized particles; evidence was that most of these vacuoles were lysosomal in origin (see Fig. 4), but that some probably arose by swelling and disintegration of melanosomes (× 7400). (b) Melanocyte from 15-day dodecandioic acid culture exhibiting similar features (× 15,000).

and, once again, the melanocytes with most numerous and most heavily melanized melanosomes were predominantly present in the cultures to which dodecandioic acid had been added. Also there was no evidence that dicarboxylic acids had a positive cytotoxic action on the melanocytes.

DISCUSSION

Before dealing with the effects of addition of dicarboxylic acids to the medium, it is as well to draw attention to some features of normal human melanocytes in tissue culture as revealed by the present investigation. Of the two types of cell present at 7–10 days it seems reasonable to assume that the rounded type represents cells present in the original epidermis, and that the spindle-shaped type represents 'younger' cells which subsequently grew in culture; even with control, non-experimental cultures, one would expect the former to contain more numerous and more heavily melanized melanosomes than the latter, and this in fact is what was observed. The presence of some cells of the spindle-shaped type in cultures at all stages, and almost exclusively at 20 days, tends further to confirm their designation as young cells just commencing melanogenesis. The predominance of this type of cell at 20 days also indicates that the survival time of the majority of the melanocytes present when the cultures were started is between 2 and 3 weeks, and appearances present at 15 days indicate that their destruction is associated with an increasing build-up in numbers of lysosomes which subsequently become vacuolated, and a degradation of melanosomes, both of themselves and within phagosomes.

The shape and internal structure of the melanosomes is also a matter of interest. The spheroidal shape of the heavily melanized organelles of the 'older' cells might be regarded as a change preliminary to their vacuolation and degradation. However, even developing melanosomes of control melanocytes were predominantly of spheroidal shape, and of granular, rather than laminar internal structure. This suggests that general culture conditions *per se*, apart from the effects of specific additives, can directly influence melanosome morphology, a matter which merits further study.

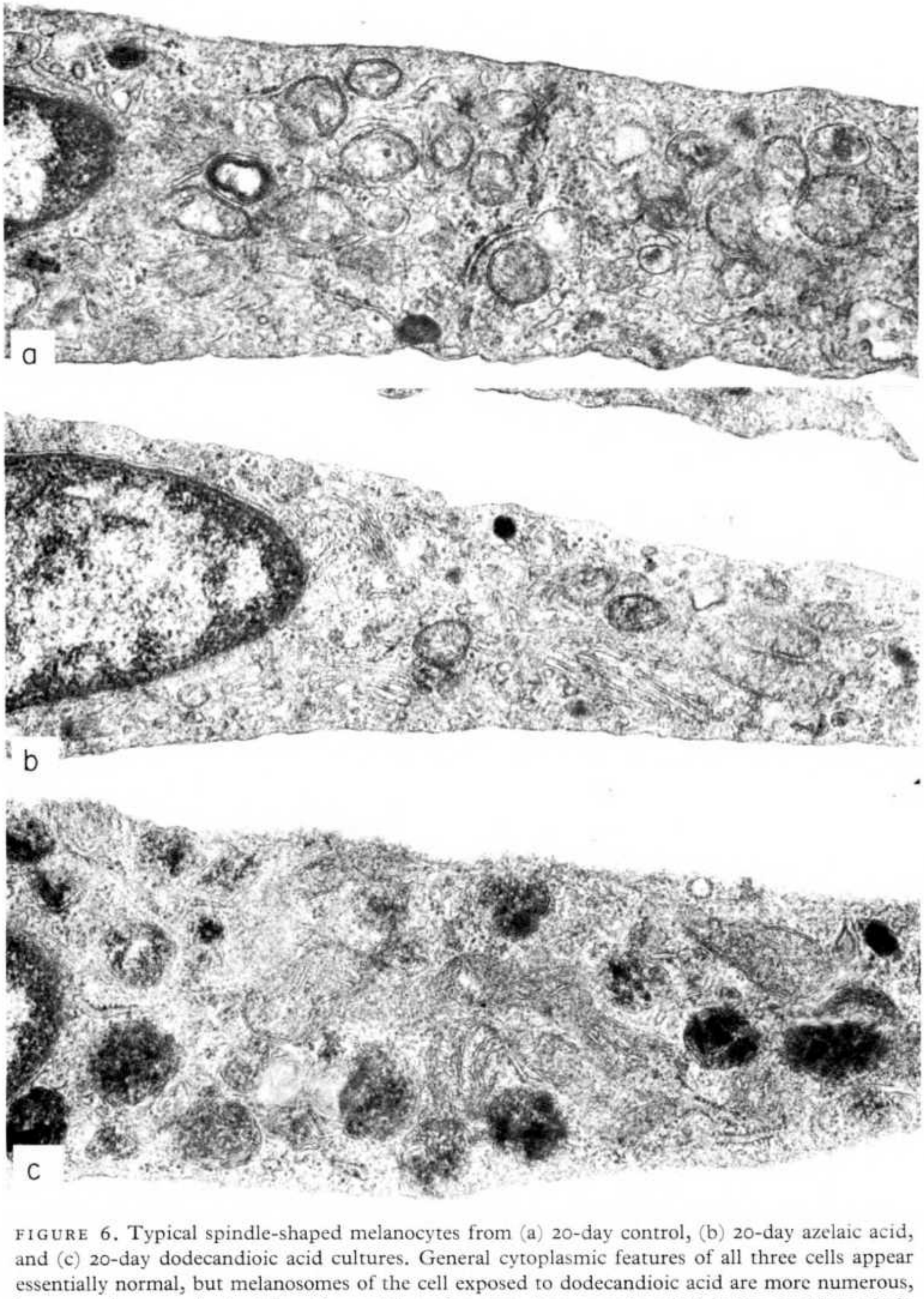

FIGURE 6. Typical spindle-shaped melanocytes from (a) 20-day control, (b) 20-day azelaic acid, and (c) 20-day dodecandioic acid cultures. General cytoplasmic features of all three cells appear essentially normal, but melanosomes of the cell exposed to dodecandioic acid are more numerous, larger, and more melanized than those of the other two; they are spheroidal in type and irregularly melanized (× 33,750).

Bearing in mind these features of normal control cells, which were also observed to more or less equal degree in the experimental cultures, it is clear from this investigation that, apart from apparently stimulating melanogenesis, the dicarboxylic acids, azelaic and dodecandioic, have no direct toxic or cytolytic effect on cultured normal human melanocytes when present in the medium in concentrations up to 200 μg/ml and for periods up to 30 days. Melanocytes can evidently grow and reproduce in their presence. These results are surprising, from a number of viewpoints. It has been clearly established that these substances inhibit tyrosinase activity *in vitro* through the mechanism of competitive inhibition (Nazzaro-Porro & Passi, 1978), and there is good evidence that they are responsible for the hypopigmentation in pityriasis versicolor (Nazzaro-Porro & Passi, 1977); they are effective agents in the treatment of a number of functional hyperpigmentary disorders (Nazzaro-Porro & Passi, 1978b) and lentigo maligna (Nazzaro-Porro *et al.*, 1979), and preliminary results show that they retard the growth rate of transplanted Harding-Passey melanoma in mice, and lead to prolongation of survival of the animals (Nazzaro-Porro *et al.*, 1978). The present results on normal melanocytes in tissue culture are exactly the opposite to what might be expected from these observations, and no plausible explanation comes readily to mind. The suggestion that conditions in tissue culture are so artificial anyway that one need not be surprised at any result, has little appeal. It could be argued that the stimulation of melanogenesis is indeed a direct, though paradoxical, result of the tyrosinase-inhibitory property of dicarboxylic acids. Thus, it may be suggested that, in competing with or binding to tyrosinase, they reduce the concentration of available enzyme, and that this in some way is 'sensed' by the synthetic centres of the cell, which proceed to produce an excess of enzyme, over-compensating for the inhibitory effect, and leading to increased melanogenesis. This is, of course, highly speculative, but some such mechanism might also account for the initial hyper-pigmentation commonly observed to precede the hypopigmentation in pityriasis versicolor.

The lack of any apparent general cytotoxic effect on normal melanocytes in culture is very puzzling in view of the clinical and histopathological observations in the conditions mentioned above. These clearly reveal that hyperactive or structurally abnormal melanocytes are destroyed by exposure to dicarboxylic acids *in vivo*, but it is not known if this is directly releated to tyrosinase inhibition, or if it is a separate effect. It may be that in the present experiments the concentration of dicarboxylic acids was sufficient to produce only a stimulatory effect upon the melanocytes, and that if it had been higher, a positive cytotoxic effect might also have been observed. However, the concentrations used seemed sufficiently high to produce all possible effects. Besides, it was found (Nazzaro-Porro *et al.*, 1979) that application of a dicarboxylic acid cream, similar to that which proved so highly effective in the hyperpigmentary disorders mentioned above, failed to have any destructive effect on the great majority of melanocytes of normal skin *in vivo*. This and the present observations could indicate that dicarboxylic acids may be cytotoxic only to melanocytes that are abnormal in some way, or at some phase of their life cycle. If this be so, their action and therapeutic value would be very different to other substances cytotoxic to melanocytes, such as hydroquinone, 4-tertiary-butyl cathecol, mercaptoamines, etc., which destroy alike the normal as well as the abnormal melanocyte (Bleehen *et al.*, 1968; Jimbow *et al.*, 1974). Evidently the mode of action of these substances requires further investigation, and the next step should be to examine their effect on melanocytes in organ cultures of normal and abnormally hyperpigmented human skin.

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Lichenoid melanodermatitis

A CLINICOPATHOLOGICAL STUDY OF FIFTY-ONE KENYAN PATIENTS WITH SO-CALLED TROPICAL LICHEN PLANUS

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SUMMARY

Fifty-one Kenyan patients with variable round plaques on the exposed parts, predominantly on the face, and characterized by central hyperpigmentation with annular hypopigmented borders, are described. The clinical and histological picture showed transitions, which ranged from lichen planus and lichenoid poikilodermatous syndromes to dermatitis. This syndrome corresponds largely with (sub-)tropical or actinic lichen planus as reported from the Middle East, where emphasis has been given mainly to a part of the described spectrum of transitions. We suggest that this syndrome should be considered as one of Pinkus' regional lichenoid syndromes which represent hybrids between lichen planus and other conditions rather than as a mere variant of lichen planus.

In Kenya, almost 1% of new patients visiting the skin clinics present with a peculiar dermatosis of the exposed parts (Verhagen *et al.*, 1968). It consists of well-defined nummular patches, mainly on the face, with a strongly hyperpigmented centre, surrounded by a striking hypopigmented annular zone (Fig. 1). The patches are only slightly raised and fairly stable from the beginning; there is no evidence of an acute stage. No counterpart of this syndrome seems to exist in the European literature but in the Middle East, lesions with an identical aspect are classified as actinic or (sub-)tropical lichen planus (Dostrovsky & Sagher, 1949; Seif el Nasr, el Zawahry & el Gohari, 1962; Katzenellenbogen, 1962; el Zawahry, 1965, 1969; Dilaimy, 1976). We have established a greater variability in the clinical and histopathological picture than is suggested by the diagnostic label.

METHODS AND MATERIALS

The criterion for inclusion in this study was the presence of patches as described above, localized on the sun-exposed parts, that could not be classified among established dermatoses (see below).

Fifty-one patients were investigated at both urban and rural clinics. A standardized questionnaire

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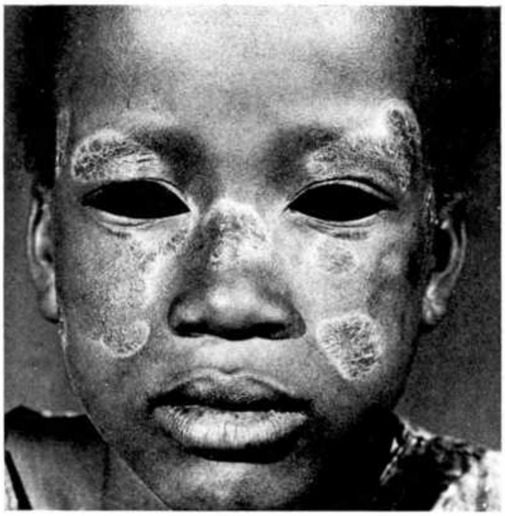

FIGURE 1. Lichenoid melanodermatitis of the intermediate type.

was used for the case history, with emphasis on the influence of sun exposure, internal diseases, diet and the use of drugs, soaps and cosmetics. The histological investigation included haematoxylin-eosin, PAS and melanin stains. Occasionally, fluorescent techniques were applied.

Fifteen patients were irradiated with a xenon arc lamp for 20 min at 70 cm distance (Orsam XBF 6000, fitted with Schott WG 6 filter of 2 mm). This light source produces a continuous spectrum from 290 nm upwards, simulating natural midday sunlight. The dose represented about twice the average minimal erythema dose for Africans. It was applied three or four times on consecutive days on the same area of the cheek.

Cases with well established dermatoses, notably discoid lupus erythematosus and fixed drug eruption, were excluded on the basis of a different appearance, course, case history and histopathology, and the immunofluorescent picture of the former.

RESULTS

It soon became apparent that the aforementioned lesions varied in aspect and should be divided into several types. Sub-groups were defined first according to the clinical and histological morphology. Subsequently additional features were considered in the light of this classification. Results will be presented in this order.

Clinical findings

The variation in appearance could best be described as a continuous spectrum, ranging between two polar types. One polar end was typified by relatively large lesions, up to 4 cm in diameter, with well circumscribed, but not sharp, margins. The hyperpigmented centre was relatively small, the larger part consisting of a broad, yellowish periphery resembling pityriasis alba. These lesions exemplified the 'eczematoid' polar end. The alternative 'lichenoid' polar end was typified by smaller, more numerous, sharply marginated, very black patches. The delicate hypopigmented edges had a bluish tinge (Fig. 2).

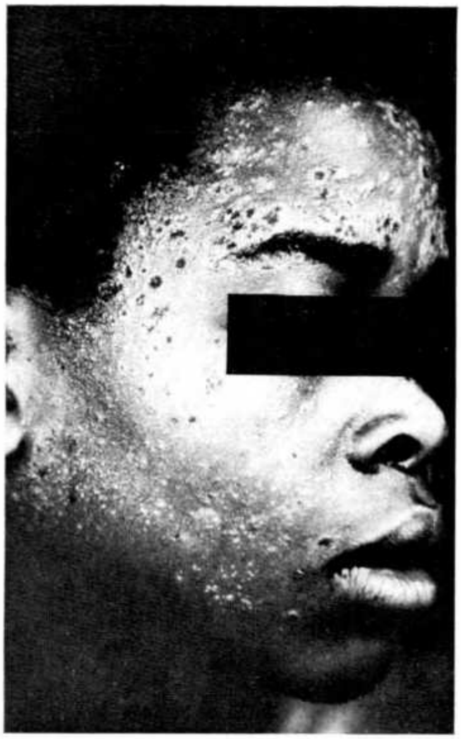

FIGURE 2. Skin lesions typifying the polar lichenoid type. Note the hypopigmented peripapular haloes.

The intermediate type was the most characteristic and consisted of sharply marginated, nummular patches 2–3 cm in diameter. The centre showed a marked hyperpigmentation with a peculiar slate-coloured tinge, which contrasted with the clear hypopigmented border, measuring 1–2 mm (Fig. 1).

In all three types various degrees of expression were observed. In the older lesions only residual abnormal pigmentation might persist. Even active fresh lesions were flat and only mildly infiltrated; they did not consist of aggregated papules.

Invariably the face was the site of predilection. Twenty-seven patients also had minor lesions on other sun-exposed parts, mostly on the extensor side of the lower arm and hand (sixteen cases) or on the neck (twelve). Only three patients showed minor lesions on non-exposed areas. The extra-facial lesions, more frequent in both polar types, were also characteristic but often differed from those on the face. On the arms patches were irregular in outline, with yellowish hypopigmentation as the predominant feature, which induced Katzenellenbogen (1962) to describe them as 'dyschromic'. The round patches on the neck showed less overt pigmentary changes but consisted of shiny, bluish, flat lichenoid papules.

Histopathology

The lesions showed a corresponding histopathological spectrum (Table 1). In the larger, mainly hypopigmented lesions the features of dermatitis predominated. The histological pattern at the other end of the clinical spectrum was 'lichenoid' in the sense proposed by Pinkus (1973). In the intermediate type, for which we adopted the name lichenoid melanodermatitis as proposed by Greppi &

TABLE 1. Correlation between clinical and histological findings

	Frank lichen planus	Close to lichen planus	Melano-dermatitis lichenoides	Poikiloderma like	Dermatitis or close to dermatitis	Inconclusive	HISTOLOGY
Lichen planus	2	4					6
Close to lichen planus		3		1			4
Intermediate		2	6	3	5	2	18
Close to eczema type			4	1		2	7
Frank eczema type				2	11	1	14
Intermediate						2	2
CLINICAL PICTURE	2	9	10	7	16	7	51

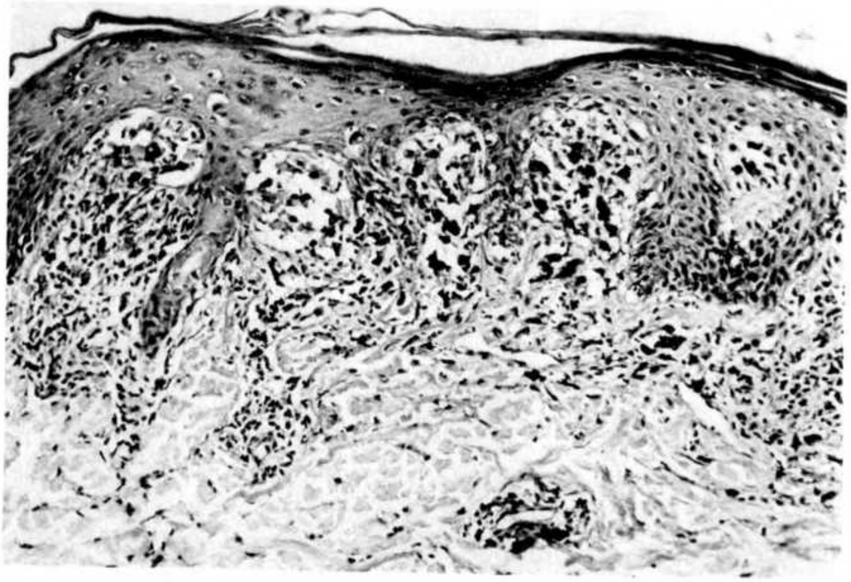

FIGURE 3. Close to lichen planus. See text.

Soustek (1966), features of a lichenoid and/or an inflammatory reaction might both be present in the same area.

The only feature in common to all types was a marked pigmentary incontinence. Abundant pigment was found in macrophages and as large extracellular clumps. Only two patients at the lichenoid end of the clinical spectrum showed unequivocally the complete histological picture of lichen planus. More common (Table 1) were cases in whom the histology was only close to classical lichen planus. A dense infiltrate, though hugging the epidermis, was less bandlike, more patchy and with a tendency to perivascular arrangement. Locally the dermo–epidermal border was as ill-defined as in classical cases. Most rete ridges had a saw tooth appearance with an irregular basal layer. On the other hand hyaline necrosis was scanty and Civatte bodies were rare, whereas liquefaction degeneration became more prominent. Moreover the thickness of the granular layer was uneven and a local flake of parakeratosis made its appearance (Fig. 3).

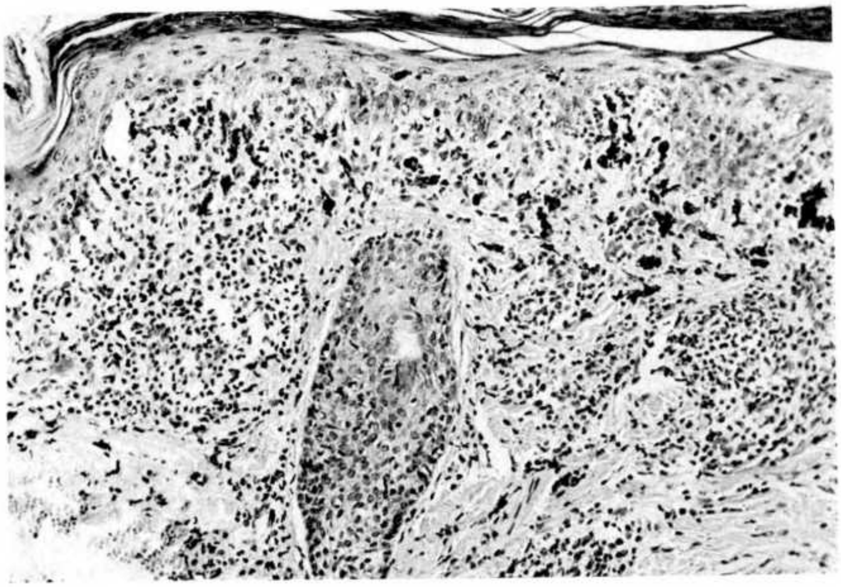

FIGURE 4. Intermediate type, note parakeratosis and the broad inflammatory zone.

From this point in the spectrum, the histological variations branched off in two directions.

(1) In some clinically less active patches the main features were severe hydropic degeneration of the epidermal basal layer, pigment incontinence and a sleeving perivascular infiltrate with only minor infiltration of the papillae. This is the histological picture of lichenoid poikilodermatous patterns (dermatosis cenicienta, Riehl's melanosis, Civatte's poikiloderma).

(2) In active lesions of the middle of the spectrum classified as melanodermatitis lichenoides (Table 1), the progression from lichenoid towards dermatitis-like features proceeded. The former persisted in the distorted dermo–epidermal interface. An occasional saw tooth rete ridge or even a small focus of hyaline necrosis was still found. However, elongated ridges and liquefaction degeneration were prominent, together with a more diffuse and perivascular infiltrate. The upper epidermis showed all the features of dermatitis, such as parakeratosis and mild spongiosis (Fig. 4). Near the end of the spectrum the histology was almost indistinguishable from dermatitis, though at the dermo–epidermal junction some densely infiltrated papillae had the form of scallops with adjoining basal cell liquefaction and minor distortion of rete ridges. It should be stressed that this histological spectrum of the facial lesions closely corresponded to the clinical appearance. In extra-facial lesions the features of dermatitis were always more pronounced.

Additional clinical features

These three clinically and histologically divergent types had, however, some features in common.

Patients tended to be younger than the average age of those consulting our clinics. Half of them were children, twenty young adults and only six over 30 years. The sex distribution was even.

All patients but two described the onset as insidious. The lesions spread gradually over a period of several months and in nine cases relapses occurred during our follow-up. The established average duration of the eczematoid variety was 8 months, and of the lichenoid variety at least 20 months.

During the follow-up the clinical picture usually remained stationary. If changes occurred the lesions tended to evolve histologically towards the lichenoid side. Ultimately they disappeared spontaneously, without leaving any atrophy.

Treatment such as corticosteroids had little or no effect. Most patients reported only slight itching. One or the other of these features contrasts with lichen planus or lupus erythematosus.

Possible contributing factors

Often patients used a great variety of industrial or home made cosmetics, oils, vaseline and soaps, several local brands having irritating properties (Verhagen, 1974). However, a matched control group used the same variety of products and no single one had been used by more than one-fifth of the patients. Neither did the standard case history establish a connection with diet, drugs, malnutrition and internal or other diseases, notably diabetes.

With regard to sunlight, twenty-six patients indicated a deleterious effect after an initial non-leading question and ten more upon closer questioning. Africans, especially children, are hardly aware of the possibility of sunlight damage. Taking this into account, the ratio of affirmative answers is nevertheless impressive and in line with the distribution of the lesions. The average patient belonged to the less prosperous strata of society and spent a considerable part of the day outdoors.

The light provocation tests produced merely an erythema and oedema, compatible with repeated high doses. Only in a single patient could a typical lesion be elicited. The average minimal erythema dose did not differ from controls.

DISCUSSION

Similar lesions as in Kenya have been reported from the Middle East, notably Egypt, Israel and Iraq. The condition was called 'actinic' (Katzenellenbogen, 1962) or '(sub-)tropical' lichen planus (Dostrovsky & Sagher, 1949; Seif el Nasr *et al.*, 1962; el Zawahry, 1965, 1969; Dilaimy, 1976). In Ethiopia, Greppi & Soustek (1966) called it 'melanodermite lichenoide'.

In all these studies patients were described with nummular or annular patches with an almost identical appearance. Closest to classical lichen planus were the description and the pictures by Dostrovsky & Sagher (1949). Apart from papular lesions, however, they also showed larger, flat and plaque-like lesions which typified this disorder in all later studies. The fine hypopigmented border, a hallmark of the disease in Kenya, was also present in most pictures from other countries, e.g. on a frontispiece photograph by el Zawahry (1967). The localization on the exposed parts and the connection with sun exposure were other common characteristics.

Our light provocation tests failed to confirm the causative influence of sunlight. However, in Africans this is found in other common skin disorders with an established adverse influence of sunlight (Verhagen *et al.*, 1968). Therefore, we attach more importance to the clinical findings. There was no connection with drugs, notably anti-malarials.

Although this condition resembles lichen planus, there were several distinguishing features. The age distribution tended towards childhood and adolescence (Katzenellenbogen, 1962; Seif el Nasr *et al.*, 1962; el Zawahry, 1965; Dilaimy, 1976), the average duration with frequent relapses was longer (Dostrovsky & Sagher, 1949; el Zawahry, 1965; Dilaimy, 1976) and most authors agree that itching was usually minimal.

In all these countries classical lichen planus existed side by side with this affection and was usually twice as common. In addition the differential diagnosis of both conditions is not difficult and transitions have never been mentioned. The histology in most Kenyan cases showed transitions between lichen planus and dermatitis, with liquefaction degeneration and pigmentary incontinence as prominent features. In the literature some confusion exists. Seif el Nasr *et al.* (1962) and Dilaimy (1976) described only the characteristic histology of lichen planus. Dostrovsky & Sagher (1949) mentioned problems in the differential diagnosis with lupus erythematosus, poikiloderma and Riehl's melanosis.

The description by Katzenellenbogen (1962), discussing the largest series of eighty-four specimens, and by Greppi & Soustek (1966) included all histological features of our spectrum. Accordingly, Katzenellenbogen (1962) stated that the histology resembled lichen planus only 'vaguely' and Greppi & Soustek (1966) termed this condition 'lichenoid melanodermatitis'. Pictures by el Zawahry (1965) which were discussed at the Eighth International Congress of Dermatology (1967) showed similar atypical features. In retrospect, this spectrum of transitions has apparently also been observed elsewhere.

Pinkus (1973) proposed the concept of 'lichenoid tissue reactions' for various conditions with basal cell damage as the essential lesion. The clinical manifestations and the epidemiology of diseases brought under this heading vary considerably. All are however characterized by a prominent pathology at the dermo–epidermal junction, e.g. liquefaction degeneration or hyaline necrosis. Lichen planus constitutes the prototype. In this disease, basal cell damage apparently results in local erosions with substitution of cells by migration (Marks, Black & Wilson Jones, 1973; Presbury & Marks, 1974). Obviously the condition we have discussed fits within this concept and seems to constitute a hybrid between lichen planus and the plaque type variant of polymorphous light eruption. Analogous hybrids exist between lichen planus and lupus erythematosus (Davies *et al.*, 1977; Romero, Nesbitt & Reed, 1977). Sunlight can induce both an inflammatory reaction as well as a basal cell damage. This has been described in actinic reticuloid (Menter, McKerron & Amos, 1974). The variable expression of both tissue responses may explain the aforementioned spectrum.

For practical reasons the name 'sub-tropical' lichen planus might be maintained, provided one realizes that both clinical and histological features are best defined as lichenoid melanodermatitis (Greppi & Soustek, 1966).

Contrary to the universal nature of lichen planus, other lichenoid reactions occur mainly in certain areas or periods. Dermatosis cenicienta among Latin Americans, lichen pigmentosus among Japanese, whereas three related conditions exacerbated by sunlight, i.e. melanodermatitis of Hoffmann and Habermann, Civatte's poikiloderma and Riehl's melanosis, were formerly more frequent in Europe (Storck, 1948).

Lichenoid melanodermatitis or sub-tropical lichen planus of the face, with minor lesions on other exposed parts, represents another distinct regional entity. Virtually all patients so far reported originate from the Middle East, East Africa and, recently, from India (Singh & Kanwar, 1976).

El Zawahry's, Katzenellenbogen's and our studies established cases from thirteen countries covering a continuous global territory from the Caspian and Mediterranean seas down to the equator. This area is characterized by hot and dry seasons. In addition a predisposition for people of moderately dark complexion is likely. In all cases from Israel, Jews were of oriental descent (Dostrovsky & Sagher, 1949; Katzenellenbogen, 1962), whereas classical lichen planus was found in co-nationals emigrated from Europe. The condition has also not been reported among Caucasians in the other cities, although there is a considerable European influx. Isolated cases from elsewhere are in fact classical lichen planus exacerbated by sunlight and rarely fit our description.

The findings in lichenoid melanodermatitis corroborate the concept of Pinkus (1973), that lichenoid features constitute a cutaneous reaction pattern rather than a single disease entity, epidemiologically modulated by such factors as sunlight or pigmentation.

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The intra-epidermal epithelioma of Jadassohn: a distinct entity

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SUMMARY

Forty cases of a distinctive acervate epidermal tumour (intra-epidermal epithelioma of Jadassohn) are described. The pathological and clinical features distinguishing it from other skin tumours are emphasized. Invasive areas were found in five of the cases, one with metastasis to a lymph node, underlining the need to differentiate this tumour from benign epidermal tumours, such as 'irritated' seborrhoeic keratosis and inverted follicular keratosis with which it has been and still is frequently confused.

Although the very existence as a distinct entity of the Jadassohn lesion has been seriously doubted (Mehregan & Pinkus, 1964; Lever, 1975), Graham & Helwig (1966) have been convinced of its distinguishing features. Similar debate exists as to the cell of origin and its biological behaviour.

We have reviewed the benign epidermal tumours in the files of The Institute of Pathology at the London Hospital in the years 1960–1976 to determine whether an epidermal tumour corresponding to that commonly associated with the name of Jadassohn exists and whether its clinical and pathological features are sufficiently characteristic for it to be acknowledged and treated as a distinct entity.

MATERIALS AND METHODS

Some cases of the Jadassohn lesion were filed specifically under that heading but since in the period under review the recognition of the Jadassohn lesion as an entity was not universal, we also selected from the files of the Institute of Pathology the benign epidermal tumours, excluding those which were obviously of viral aetiology and those derived from adnexae.

A total of 582 cases coded as benign epidermal tumours were reviewed and these included five cases separately indexed as Jadassohn lesions. Sections stained with Ehrlich's haematoxylin and eosin were examined in each case and the features of the tumours systematically recorded. Selected cases were stained with alcian blue, PAS with and without diastase treatment, alcian blue/PAS, and Schmorl's stain for melanin. Clinical information in the Jadassohn cases was also recorded.

* This work was performed while the authors were employed in the Department of Morbid Anatomy, Institute of Pathology, The London Hospital, London E1.

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PATHOLOGICAL AND CLINICAL FEATURES

The bulk of the 582 cases reviewed were seborrhoeic keratoses with a few smaller groups including inverted follicular keratoses, but it became clear on careful analysis that forty cases showed distinctive pathological features. These forty cases corresponded in many ways to the few existing descriptions of the Jadassohn lesion.

Of the forty patients (thirty-nine white Europeans and one Indian), twenty-six were female and thirteen male, there being one in which the sex of the patient was not specified. Their ages ranged from 34 to 75 years with a mean of 59·4 years.

The lesions were reported to have been present for periods ranging from 3 months to 50 years. In some cases the duration of the lesion was vague or not available but was usually said to have been present for many years.

Clinically, the lesions were described as flat, slightly elevated plaques, with an irregular outline and a scaly verrucous surface (Fig. 1). From the retrospective descriptions, it seems that many resembled rather flat seborrhoeic keratoses.

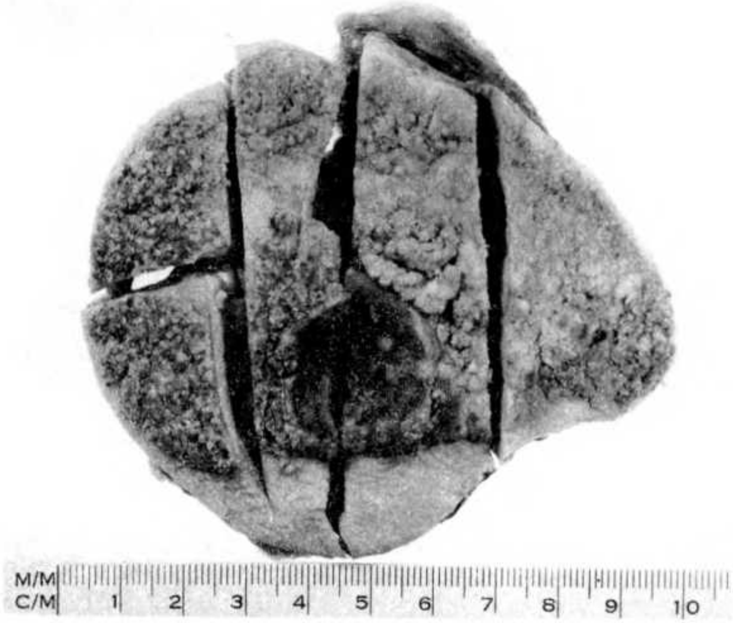


FIGURE 1. The macroscopy of a large Jadassohn lesion after its initial pathological dissection demonstrating its verrucous scaly surface and a roughly circular ulcer which is one of the poorly differentiated squamous carcinomas described in the text.

Their size ranged from 0·4 to 10 cm with a mean of 1·94 cm. These figures mask a marked variation in size between different parts of the body. There were eleven tumours on the head and these ranged from 0·4 to 2 cm with a mean of 0·68 cm. Thirteen tumours on the trunk ranged from 0·5 to 10 cm with a mean of 2·0 cm. Four tumours from the upper limb had a mean diameter of 1·5 cm and a range of 0·4 to 2 cm and the mean for the eleven tumours on the lower limb was 1·9 cm ranging from 0·5 to 8 cm. The site of one tumour with a diameter of 10 cm was not specified.

Corresponding with the plaque-like scaly macroscopic appearance, it was seen microscopically that

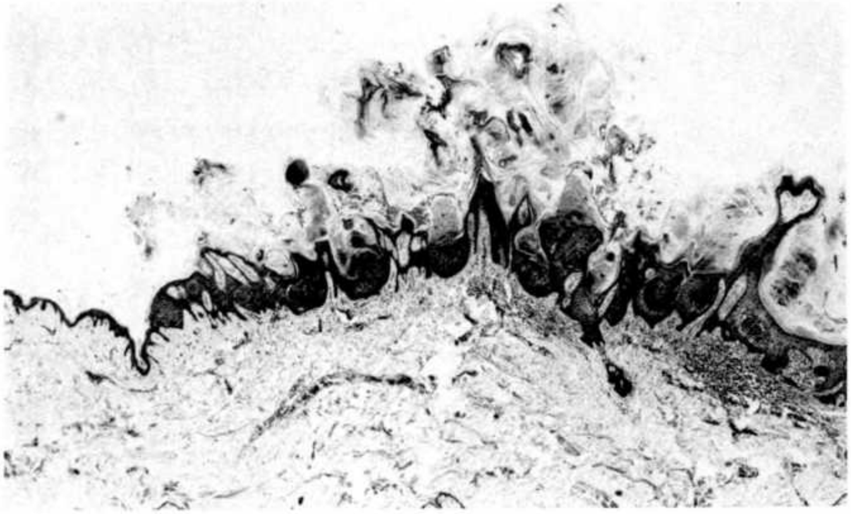


FIGURE 2. A general view of the edge of a Jadassohn lesion showing normal skin on the left and the verrucous and acervate acanthosis of the tumour (× 20).

the tumours were composed of acanthotic epidermis thrown into papillary projections and intervening slight down-growths (Fig. 2). Irregularly but frequently along the length of the tumour, distinct nests of cells are present within the epidermis (Fig. 3). These are found both in the projections and the depressed parts of the epidermis. Parakeratosis often overlies and appears in continuity with the nests (Fig. 4), while the rest of the area of the lesions is markedly hyperkeratotic. The nests have

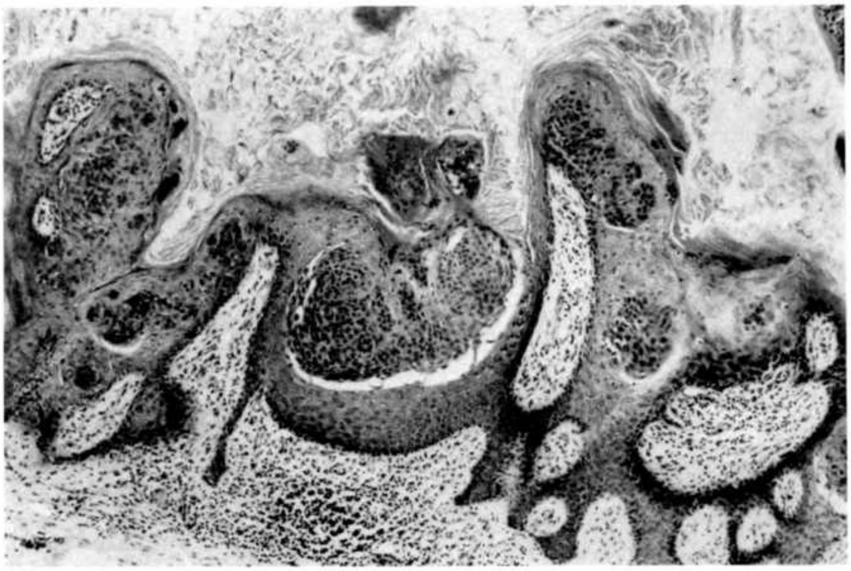
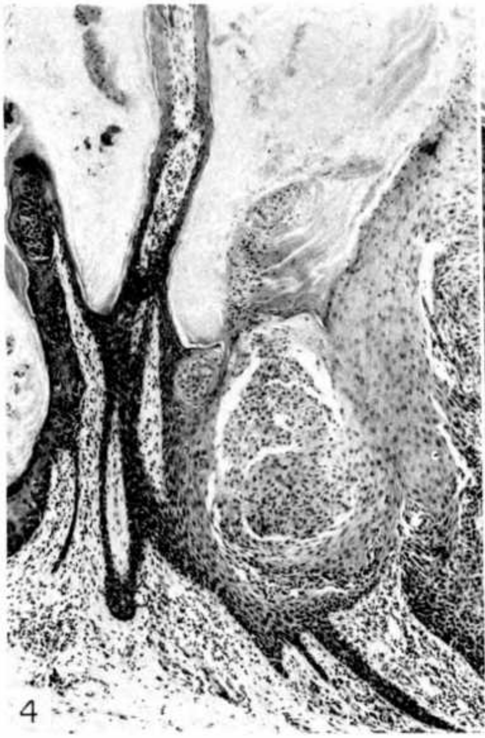


FIGURE 3. The papillary epidermal projections with irregularly distributed intra-epidermal nests are shown. One larger nest has a cleft on its deep and lateral margins (× 80).



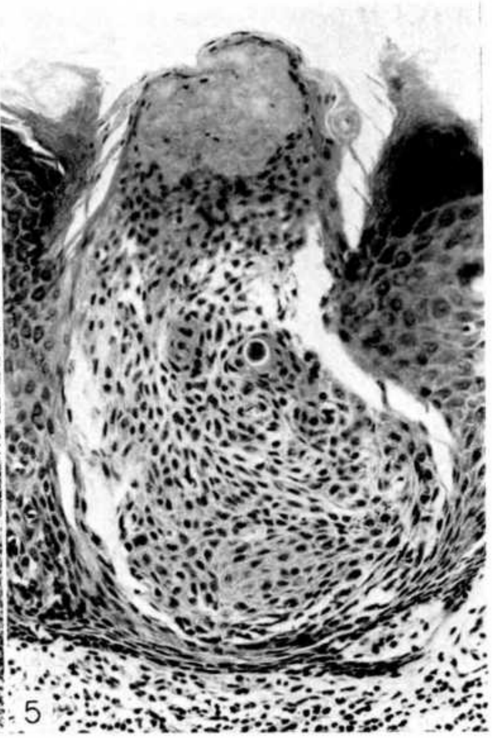


FIGURE 4. An intra-epidermal nest is partially separated from the surrounding verrucous epidermis by clefts but is continuous with the focus of parakeratosis in the overlying stratum corneum. Some clefts extend into the centre of the nest, some of the cells of which are dyskeratotic (× 80).

FIGURE 5. The squamoid morphology of the intranidal cells is apparent as are also dyskeratoses (e.g. centre field) and mitoses (e.g. bottom right) (× 200).

several very characteristic features (Fig. 5). They are situated completely within the epidermis, a rim of cells being constant between the nest and the dermo–epidermal junction and often between it and the stratum corneum. Commonly the nest is situated so that its superficial aspect is in continuity with the stratum corneum without an intervening granular layer. The stratum corneum is usually parakeratotic at that point. In these most typical nests there is no separation of cells at their superficial border but often a separation amounting to a cleft is present around the deep and lateral margins (Fig. 3). In those nests apparently in the mid-epidermis (but possibly only tangential cuts through typical superficial nests), the cleft between nest and surrounding acanthotic epidermis can be circumferential. Finer irregular clefts between cells or clumps of cells may extend towards the centre of all nests. The cells have an eosinophilic cytoplasm with relatively inconspicuous intracellular squamous bridges. The appearance of the cells in the nest is that of keratinocytes with a spectrum of maturation from undifferentiated cells to keratinizing cells. They are not arranged in maturing concentric whorls but irregularly in a loose disorderly manner accentuated when clefts are present. Mitoses and individually keratinizing or dyskeratotic cells are found commonly within the nests (Fig. 5). In small lesions of short duration they are less common. In other lesions they are present in large numbers, together with marked cellular pleomorphism; these features are strongly suggestive of *in situ* malignancy and this is borne out by the association with frankly invasive areas in other parts of some of these

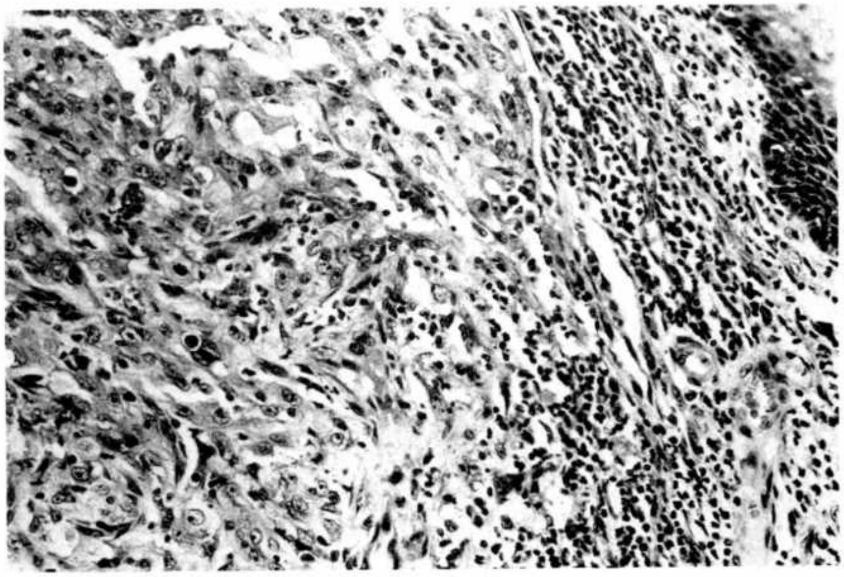


FIGURE 6 The invasive poorly dfferentiated squamous carcinoma corresponding with the macroscopic ulcer of Fig. 1. Overlying epidermis is seen (top right) (× 200).

tumours. Mitoses and dyskeratoses within the nest are so constant that their absence in all the nests of a lesion must call the diagnosis into question.

In one case, an Indian, there was particularly prominent incorporation of melanin into and around the cells of the nests and dermal histiocytes but the lesion was clearly typical in every other way and readily distinguished from malignant melanoma.

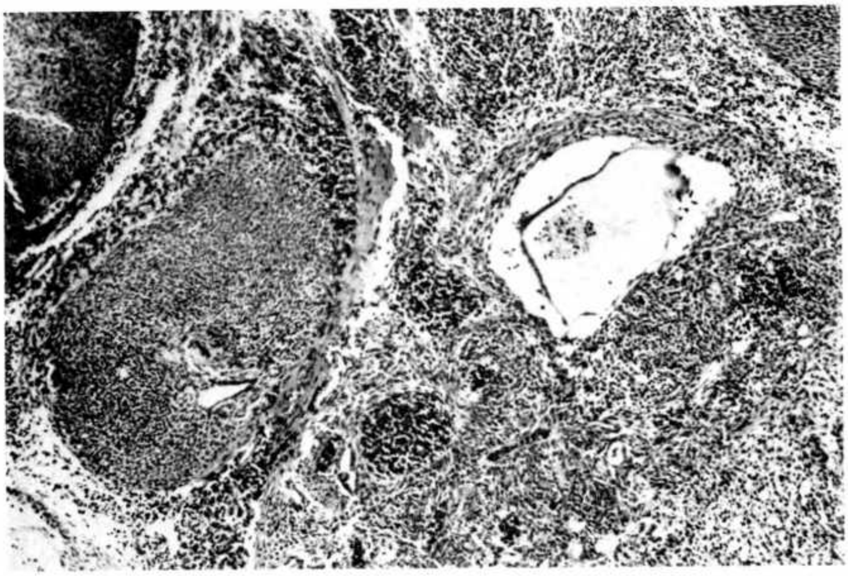


FIGURE 7. These two tubular structures surrounded by smaller uniform cells, suggesting sweat duct origin, were seen within an early invasive squamous carcinoma (× 80).

The epidermis between nests of the Jadassohn lesion is acanthotic and hyperkeratotic but does not show disorderly pattern, atypia, increased mitosis or dyskeratoses. The superficial dermis underlying these tumours is often lightly infiltrated by lymphocytes (Fig. 3), but there are no other dermal changes.

Five of this series of patients with Jadassohn tumours had invasive areas, in most cases confined to the superficial dermis. However, one patient had a large Jadassohn lesion over the scapula, 9 cm in diameter, which included an ulcerating deeply invasive poorly differentiated pleomorphic squamous carcinoma (Fig. 6) which had metastasized to an axillary lymph node. The same Jadassohn tumour also included other multiple separate areas of early invasive squamous carcinoma. In addition this lesion included one intra-epidermal area with smaller uniform cells and occasional ductular structures suggesting an origin from ducts of sweat glands (Fig. 7). Diastase resistant PAS positive material was present in these ducts.

One of the invasive areas of one of the other four cases had similar appearances with a mixture of clear squamoid cells and occasional small ducts with the same PAS-positive material in the ducts, suggesting an origin from the acrosyringium.

Two other cases had areas of invasion of poorly differentiated squamous carcinoma and the invasive tumour in the fifth case was indistinguishable from a basal cell carcinoma. The Jadassohn lesion in the epidermis in this last case had some of the features of the hidroacanthoma simplex described by Smith & Coburn (1956).

So of the total of forty cases with Jadassohn tumours, three had invasive squamous carcinomas, one with a metastasis to a regional lymph node. Two other cases had invasive tumours of low-grade malignancy or limited invasive potential.

DISCUSSION

The term Borst-Jadassohn lesion has been used by different authors in different senses. Borst (1904) described intra-epithelial spread of malignant cells from an adjacent carcinoma but this is clearly not what is generally intended nowadays by the term. Jadassohn (1926) in a review of basal cell carcinomas did describe primary epidermal clustering in one case, although he thought he was describing a lesion similar to that of Borst. It is doubtful whether Borst or even Jadassohn merit the eponymous use of their names in association with the tumour described here. Most subsequent authors have entirely discounted the existence of this entity (Mehregan & Pinkus, 1964; Lever, 1975), believing that the lesions described as 'Jadassohn epitheliomas' are varieties of seborrhoeic keratosis, Bowen's disease or 'hidroacanthoma'. On the other hand, Haber (1958), Nicolis, Capetanakis & Panas (1971) and Graham & Helwig (1966, 1972) who documented a large series of cases, gave convincing accounts of its pathological identity. They adopted the name intra-epidermal epithelioma of Jadassohn, a description which we are retaining for convenience and to avoid further confusion.

Our study of epidermal tumours enables us to conclude confidently that the Jadassohn tumour is a distinct entity. Its most important features are the presence of multiple intra-epidermal clusters of cells superimposed on a verrucous acanthotic hyperkeratotic and parakeratotic area of epidermis. The cellular clusters of this tumour are distinguished from the grouping of cells occurring in other skin tumours in that all proliferation occurs dominantly within the cluster. This is indicated by the frequent presence of intranidal mitoses and the immaturity and disorder of the cells. This contrasts with the concentric eddies and whorls of mature squamous cells which can be seen in inverted follicular keratoses and some so-called 'irritated' seborrhoeic keratoses. Mitoses in these other tumours are present in the intranidal areas and hardly ever in the mature cells of the nests. In the tumour which is the subject of this study, the characteristic and distinctive pattern is that of disorderly centrifugal growth, focused within clusters, a microscopic pattern which can be consicely termed acervate (L *acervatus*, growing in clusters or heaps).

Not only is this acervate pattern of growth different from the eddying and whorling phenomena of other tumours, but generally the nests of this acervate tumour are larger than the 'eddies' of other tumours. Their site in the superficial epidermis, continuity with the stratum corneum and circumferential and intranidal clefts are quite distinct from the generally more deeply situated eddies with variable and irregularly distributed clefts of inverted follicular keratosis; and the less numerous, irregularly situated squamous whorls lacking clefts of irritated seborrhoeic keratoses.

Some authors (Goltz, Fusaro & Sweitzer, 1957; Lever, 1975) have stated that this tumour has no connotation of premalignancy. Mehregan & Pinkus (1964) described those epidermal lesions which showed a nesting phenomenon and pointed out that most were seborrhoeic keratoses. Among the others were some malignant conditions such as Bowen's disease and malignant melanoma. They also mentioned hidroacanthoma as a cause of epidermal nesting but they did not acknowledge the peculiar and distinct acervate form of growth we have described. They thus did not appreciate its premalignant potential. However, Graham & Helwig (1966) have reported frank carcinoma occurring in seventeen cases of intra-epidermal epithelioma. Four other cases of carcinomatous change have been recorded (Berger & Baugham, 1974; Haber, 1958; Tappeiner & Pfleger, 1964; Mitchell, 1975). Smith & Coburn (1956) have also reported two cases of malignant change in hidroacanthoma simplex, one of them occurring in a sub-variety which they call verrucous and which appears indistinguishable from the acervate tumour described in this paper. These reports of invasive carcinoma are listed, together with the invasive tumours from the five patients in our series, in Table 1.

TABLE 1. Invasion in intra-epidermal epithelioma

	Invasive tumour	% of total	No. with metastases	Reported type of carcinoma	Survival
Graham & Helwig (1966, 1972)	17	8·5	1	Squamoid or basaloid	One related death
Berger & Baugham (1974)	1	Not given	0	Squamous	4 years—death unrelated
Haber (1958)	1	Not given	0	Undifferentiated with alveolar pattern	Not given
Tappeiner & Pfleger (1964)	1	Not given	0	Poorly differentiated squamous	Not given
Mitchell (1975)	1	Not given	0	Squamous	Not given
Smith & Coburn (1956)	1	Not given	0	Keratinizing squamous	Not given
Cook & Ridgway (1979)	5 patients	12·5% had invasive tumours, 7·5% had invasive squamous carcinoma	1	Three poorly differentiated squamous carcinomas plus multifocal early invasive squamous cell carcinoma in one patient. Two tumours of sweat duct origin. One basal cell carcinoma.	No related deaths

Graham & Helwig noted that 8·5% of the patients had invasive areas within their epidermal tumours while we found 12·5%. If the cases of low-grade malignancy in our series are excluded, 7·5% of the patients had invasive squamous carcinoma. Haber (1958), Smith & Coburn (1956) and Graham & Helwig (1966) also remarked on evidence of *in situ* malignancy in these tumours. The amount of pleomorphism, mitotic activity and dyskeratosis shows considerable variation and it is impossible to indicate one point in the spectrum where *in situ* malignancy begins but certainly it is our impression that one end of this spectrum can clearly be regarded as intra-epidermal carcinoma.

The evidence then that these acervate (Jadassohn) tumours are potentially and often frankly malignant is strong but it is worth noting that they have only twice been noted to metastasize and only once have been related to the death of the patient. In the absence of metastases, wide local excision should be adequate treatment.

In most reported cases, the carcinomas are a variety of squamous carcinoma although Haber (1958) described his case as undifferentiated with an alveolar pattern. We also found three squamous carcinomas and in one patient multifocal areas of early invasive squamous carcinoma. We also found one basal cell carcinoma and two tumours with squamous and ductular areas suggesting an origin from acrosyringium. Smith & Coburn (1956) in using the term hidroacanthoma simplex clearly thought that their tumours arose from the acrosyringium but noted two patterns, the verrucous form of which we think is the same as the Jadassohn lesion. Graham & Helwig (1973) also regard them as epidermal tumours with potential for divergent differentiation probably arising from the epidermal part of the sweat duct. The acervate pattern of growth plus the presence of sweat duct-like differentiation in two of our series may constitute support for the origin from the acrosyringial keratinocytes but the lesion appears distinct from the 'plane' form of hidroacanthoma simplex described by Smith & Coburn (1956) and Mehregan & Levson (1969).

Other authors (Mehregan & Pinkus, 1964; Goltz *et al.*, 1957; Lever, 1975) regard the Jadassohn clustering phenomenon as a change occurring in seborrhoeic keratosis. However, the features of these acervate tumours are quite distinct even from the irritated seborrhoeic keratosis (Lund, 1971) which has cell clusters but of mature squamous type. The latter tumour, inverted follicular keratosis and the 'plane' form of hidroacanthoma are the only lesions with which the Jadassohn tumour may easily be confused.

The macroscopic features of these acervate tumours are very similar to those of seborrhoeic keratosis, but generally appear flatter geographical areas and less polypoid. The size of the tumour varies with site, so that those on the face and other presumably more frequently inspected areas are generally small (less than 1 cm), whereas those on the trunk or limbs are often much larger. There appears to be no significant difference in the age and the sex distribution of those tumours which become invasive and those which do not. The markedly higher incidence in males previously reported (Graham & Helwig, 1972) is not confirmed. The duration of the lesions before carcinoma supervenes is reported to be many years and this is also the case in our series.

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Reversible binding of 5- and 8-methoxypsoralen to human serum proteins (albumin) and to epidermis *in vitro*

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SUMMARY

Binding of the two photosensitizers, 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP), to serum proteins and to epidermis was measured. 8-MOP binds to serum proteins with an apparent dissociation constant (K_d) of 4×10^{-5} M. Under conditions of oral therapy, serum concentrations of the photosensitizer 2 h after administration are usually in the range of 100–1000 ng per ml serum. In this concentration range, 75–80% of the drug was found to be reversibly bound to serum proteins. 5-MOP shows a higher binding affinity to serum proteins and 98–99% of the drug is protein bound. The binding of both psoralen derivatives appears to take place mainly to serum albumin. 5-MOP and 8-MOP bind to different and non-interacting sites on serum proteins and the binding of the one has no effect on the binding of the other methoxypsoralen.

Both photosensitizers bind reversibly to human epidermis. 8-MOP concentration in the epidermis is increased by ten to twenty fold compared with the equilibrium buffer. 5-MOP shows a higher binding affinity, resulting in a higher tissue concentration of the photosensitizer. As in serum, the two drugs appear to be bound in the epidermis to independent and non-interacting sites.

No binding competition was found between the two methoxypsoralens and hydrocortisone, fluocinonide and acetyl salicylic acid, either in serum or in epidermis, using up to 1000 fold higher concentrations as compared with those of 5-MOP and 8-MOP.

The increasing employment of 8-methoxypsoralen as a photosensitizer for the treatment of various skin disorders, such as psoriasis or vitiligo, emphasizes the urgent need to know and understand its pharmacological behaviour and mode of action. The recently introduced analytical methods for

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determining 8-MOP serum levels have clarified the question of serum kinetics of the photosensitizer and have shown a serum half-life time of 1·5–2 h. (Ehrsson et al., 1977; Gazith & Schaefer, 1977; Steiner et al., 1977; Wilkinson & Farber, 1977; Gazith, Schalla & Schaefer, 1978; Schmid & Koss, 1978). The next step in attempting to elucidate the drug's pharmacological behaviour is, evidently, to correlate serum levels with the bioavailability of the drug, both in serum and in the target tissue itself.

Binding of a drug to serum proteins will determine the bioavailable drug concentration in the serum (that concentration which is free to diffuse and to equilibrate with the skin or with other tissues). Binding of the photosensitizer to cellular components will, on the other hand, determine its tissue concentration as compared with serum levels, as well as its concentration at various locations within the tissue itself, and may further lead to a partial compartmentalization of the drug within the tissue or the cells. The reaction of the photosensitizer could be controlled (or modified) to some extent by such tissue binding, since the interaction with specific cellular components will be influenced by the drug's concentration at various cellular locations. Although the drug may interact with a certain component with high affinity when investigated under *in vitro* conditions, the *in vivo* reaction may yield different characteristics due to the influence of such cellular (or tissue) binding.

In the work presented below, 8-MOP and 5-MOP binding to serum proteins was investigated, as well as their binding to human epidermis. 5-MOP is a structural isomer of 8-MOP and was shown by Pathak & Kraemer (1969) to interact with nucleic acids in a manner similar to that of 8-MOP. 5-MOP is known as a photosensitizer and was shown to sensitize skin after local or oral administration. In a recent investigation, Hönigsmann et al. (1979) have employed 5-MOP as a photosensitizing drug for the oral photochemotherapy of psoriasis and have found it to be at least as effective as 8-MOP.

In all the studies reported here, care was taken to avoid direct illumination of the photosensitizers and the binding measured was in all cases a reversible process, not associated with a photochemical reaction.

MATERIALS AND METHODS

Crystalline 8-methoxypsoralen (Meladine) was obtained from Dr K.Thomae GmbH (Biberach, Germany). Crystalline 5-methoxypsoralen was a gift from Laboratoires Goupil S.A. (Rungis, France). Human serum albumin (Fraction V) was purchased from the Sigma Chemical Co., St Louis, Mo., U.S.A. Radioactive labelling of the two methoxypsoralens was carried out by the Radiochemical Centre, Amersham, employing catalytic tritiation.

The specific activity of the tritiated drugs was 4 mCi/mg for 8-MOP and 1 mCi/mg for 5-MOP. Before use, each substance was diluted with unlabelled drug to yield the desired specific activity. Usually, 1×10^4–2×10^4 c.p.m./ml of the labelled substance were employed (2–10 ng/ml), together with concentrations of the unlabelled substance in the range of 0·1–200 μg/ml. The purity of both labelled and unlabelled drugs was routinely checked by thin layer chromatography using a benzene acetone (9:1) solvent (Gazith & Schaefer, 1977). No tritium exchange was observed for the duration of the binding experiments with any of the employed substances.

Dialysis experiments were carried out in small dialysis cells, having a volume of 0·1 ml on each side of the dialysis membrane. To ensure the thorough mixing of the cell contents, a glass pearl of 2 mm diameter was placed in each half of the cell, which was then rotated slowly (30–40 r.p.m.) for the duration of the experiment. Prior to use, the dialysis membranes (Union Carbide, Visking tubes) were washed with 1% acetic acid, followed by a second wash with 5% $NaHCO_3$. At the termination of the dialysis time, 0·05 ml samples were withdrawn from each side with a micro-syringe and radioactivity was measured in a liquid scintillation counter (Beckman Instruments, LSA 230). For all experiments involving dialysis of serum, 0·1 M of $NaPO_4$ buffer, pH 7·2, was employed as the dialysis buffer. No binding of the two photosensitizers to the dialysis membrane was observed.

To present the results of the binding experiments, the binding was described by the simplified, straight line equation of Scatchard (1949), as described by Colowick & Womack (1969) and Rodbard & Feldman (1975): $B = n - K_d \times (B/F)$, where B = concentration of the bound ligand; K_d = dissociation constant; F = concentration of the free ligand; and n = number (or the concentration) of the binding sites. When a plot of B against the ratio B/F is made, the slope of the line is equal to $-K_d$ and the value of n can be obtained from the intercept on the Y axis. When B is expressed as moles of bound ligand per mole protein, the value of the Y intercept gives directly the number of binding sites per protein molecule. In cases where the binding data did not allow the construction of such a graph, the binding was expressed as per cent of the bound drug from the total drug concentration.

To measure competition in binding between two different drugs, the binding of a constant concentration of the first (labelled) substance was measured in the presence of various concentrations of the second (unlabelled) drug, the concentration of which was varied over a range of 10^5. In these cases, a concentration of about 10^{-8} M of the labelled drug was employed, while the concentrations of the unlabelled substance varied between 10^{-9} and 10^{-4} M. Competition in binding is expressed in this case as a reduction in the binding of the labelled photosensitizer caused by the increasing concentrations of the unlabelled drug.

Aqueous solutions of 5- and 8-MOP were prepared in two ways:

(1) By diluting concentrated ethanolic solutions of known concentrations of the psoralen derivatives into 0·1 M phosphate buffer, pH 7·2 (or serum), yielding a final ethanol concentration of 1–4% in the serum and methoxypsoralen concentrations of up to 230 μg/ml.

(2) To avoid the addition of ethanol to serum, the latter was stirred for several hours with the dried (diluted and crystallized) substance of a known specific activity, followed by centrifugation to remove

TABLE 1. Binding of 5-MOP and 8-MOP to epidermis. Incubation of the epidermis was in 2 ml of 0·2 M phosphate buffer, pH 7·2, and was continued for 36 h at 4°C. Methoxypsoralen concentrations were measured in the solution before introducing the epidermis and after its removal, the difference being that amount of the drug which is bound by the tissue

mg epidermis	8-MOP in solution (M)	8-MOP in epidermis (M)
4·35	$2·77 \times 10^{-5}$	$29·0 \times 10^{-5}$
5.09	$2·61 \times 10^{-5}$	$55·9 \times 10^{-5}$
4·30*	$2·64 \times 10^{-5}$	$56·8 \times 10^{-5}$
4·65*	$2·81 \times 10^{-5}$	$19·8 \times 10^{-5}$
mg epidermis	**5-MOP in solution (M)**	**5-MOP in epidermis (M)**
0·84	$1·79 \times 10^{-5}$	$617·9 \times 10^{-5}$
0·81	$1·77 \times 10^{-5}$	$664·8 \times 10^{-5}$
1·23*	$1·72 \times 10^{-5}$	$476·1 \times 10^{-5}$
0·50*	$1·84 \times 10^{-5}$	$907·6 \times 10^{-5}$

* Epidermis was separated by stretching.

the undissolved drug. The concentration of the dissolved substance was determined from radioactivity measurement, based on the known specific activity of the drug. Concentrations of up to 200 μg 8-MOP per ml serum could be reached in this way.

In comparing these two methods, no effect of the added alcohol on the binding of the two methoxypsoralens to serum proteins or to epidermis could be detected.

Epidermis was separated after heating 1-day-old cadaver skin samples to 52°C for 1–2 min. To check for possible artifacts resulting from the heating, epidermis samples from the same skin were separated by stretching and were employed together with the heat-separated epidermis (Table 1). When binding to epidermis was measured, a weighed sample (0·5–10 mg) was placed in 2 ml of 0·2 M phosphate buffer, pH 7·2, containing the desired drug concentration, and was left to equilibrate with the solution for 36 h at 4°C with continuous, gentle, stirring. Drug concentration in the solution was measured before introducing the epidermis sample and after its removal the difference consisting of that amount of the drug taken up by the tissue. Since in both cases a constant buffer volume was taken for measurement and the concentration of the photosensitizer was determined, no correction is necessary for possible swelling of the epidermis or for buffer absorbed by the tissue. A specific density of 1·20 g/cm^3 for the epidermis was determined by short time centrifugation in a series of glucose solutions of known densities (Weast, 1977) and determining that density which would just prevent the sedimentation of the epidermis sample. Using this value the tissue volume could be estimated (1·0 μl volume for 1·2 mg epidermis) and the substance concentration in the epidermis could then be calculated and compared with the photosensitizer concentration in the buffer.

RESULTS

Binding to serum proteins

In preliminary dialysis experiments it was found that the time required to reach equilibrium with 5-MOP or 8-MOP as the dialysable component is much longer than expected. As can be seen in Fig. 1,

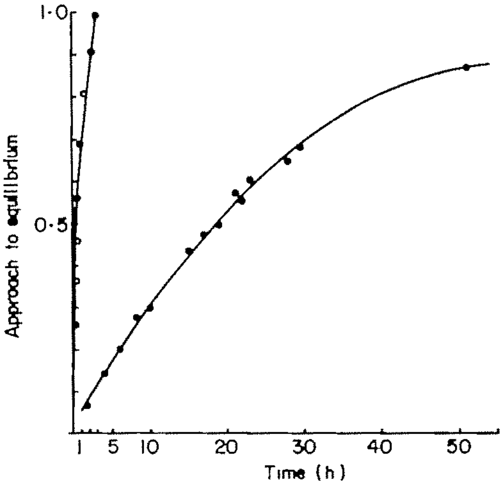

FIGURE 1. Comparison of time course of equilibrium dialysis of glucose and 5-MOP. Tritium labelled 5-MOP and ^{14}C glucose were added to serum and were dialysed against unfortified serum. Dialysis was performed in small dialysis cells (0·1 ml on each side of the membrane) at 4°C. At various times, samples were taken for measurement (each time a different cell). The results are expressed as the approach to equal distribution of the labelled substance, where 1·0 represents complete equilibrium of 5-MOP (glucose) between the two sides of the dialysis membrane. (●) 5-MOP; (○) glucose.

3 h were sufficient to reach 99% of equal distribution with glucose, but only 86% of equilibrium was reached with 5-MOP after 51 h of dialysis at 4°C. Raising the temperature to 20°C (increased molecular movement) or employing a 1/10 diluted serum (decreased viscosity) could shorten the time required to reach equilibrium by 20–40%, but in all cases dialysis times of 36 h or longer were employed in order to attain dialysis equilibrium.

To study the binding of 8-MOP to serum proteins, concentrations of 10 ng to 200 μg/ml of the former were employed (5×10^{-8}–1×10^{-3} M). The results of these experiments are presented as a Scatchard type plot in Fig. 2a. From the slope of the line, a dissociation constant of 4×10^{-5} M was calculated. When human serum albumin was employed for the binding studies instead of serum (Fig. 2b), a K_d of $3{\cdot}0 \times 10^{-5}$ M was obtained and 0·7 binding sites per protein molecule were calculated from the Y intercept.

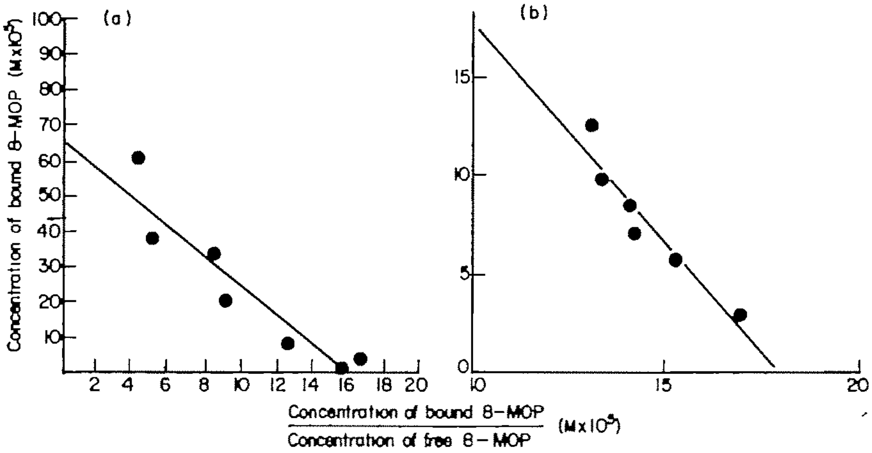

FIGURE 2. Dissociation constant determination of 8-MOP. (a) Labelled 8-MOP was added to serum and was dialysed at 10°C for 50 h against 0·2 M phosphate buffer, pH 7·2. (b) Dialysis of 65 mg/ml of human serum albumin against phosphate buffer.

When binding of 5-MOP to serum proteins was measured, a constant binding of 98–99% was observed for all concentrations in the range of 5 ng to 230 μg/ml serum (Fig. 3). Attempting to construct a Scatchard plot from such data, a vertical line (with a slope of infinity) is obtained for the concentration range used here. Since the B/F ratio remained constant at all concentrations used, no dissociation constant could be directly calculated for 5-MOP. As with 8-MOP, when the binding to human serum albumin was compared with the binding to unfractionated serum proteins, a similar binding was observed, which remained constant over the whole concentration range employed.

Competition binding

To investigate for a possible identity of the binding sites for both 5-MOP and 8-MOP, the binding of tritiated 8-MOP to serum proteins was measured in the presence of various concentrations of unlabelled 5-MOP. The results, presented in Table 2, show that no meaningful reduction in the binding of 8-MOP is observed when 5-MOP concentrations are increased up to 2000 fold molar excess over the 8-MOP concentration. As a control, the binding of labelled 8-MOP was measured as a function of the increasing concentrations of the unlabelled drug (Fig. 4). The reduction in binding of the labelled substance with increasing concentrations of the unlabelled 8-MOP is clearly seen. The

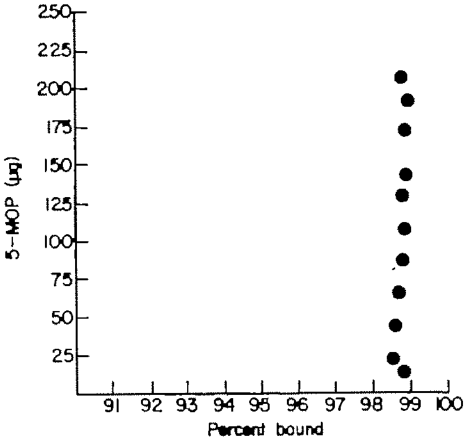

FIGURE 3. Binding of 5-MOP to serum proteins. Dialysis of 5-MOP was performed as described for 8-MOP, and was allowed to proceed for 60 h. Results are expressed as per cent of the bound drug from the total drug concentration.

TABLE 2. Competition in binding to serum proteins between tritiated 8-MOP and unlabelled 5-MOP. 8-MOP concentration is maintained constant at $8{\cdot}8 \times 10^{-8}$ M

5-MOP (M)	Bound 8-MOP (% of total)
—	77·6
—	76·4
$0{\cdot}69 \times 10^{-4}$	81·9
$1{\cdot}16 \times 10^{-4}$	74·5
$1{\cdot}86 \times 10^{-4}$	77·8
$2\;33 \times 10^{-4}$	85·4

conclusion reached on the basis of these experiments is, therefore, that these two methoxypsoralens are bound to serum proteins at different and non-interacting sites.

Competition binding to serum proteins between either one of the two photosensitizers and other drugs could be therapeutically significant, since such binding competition could change the concentration of the free, bioavailable photosensitizer and thus lead to side-effects related to the unexpected increase in the effective (not protein bound) drug concentration. Such binding competition was looked for, using three frequently employed drugs: hydrocortisone, fluocinonide and acetyl salicylic acid. No effect of these drugs on the binding of either 5-MOP or 8-MOP was observed, employing methoxypsoralen concentrations of 5×10^{-9} M and concentrations of 10^{-9}–10^{-3} M of these three drugs.

Binding to epidermis

Binding of the two psoralen derivatives to epidermal structures was measured in the absence of exciting light. Epidermis samples were incubated in 0·2 M phosphate buffer, pH 7·2, fortified with tritiated

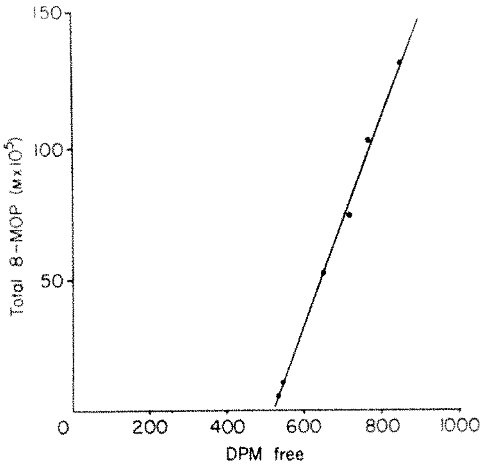

FIGURE 4. Binding competition to serum proteins between labelled and unlabelled 8-MOP. To a constant concentration of tritiated 8-MOP in serum (10^{-7} M), various concentrations of unlabelled 8-MOP were added. After reaching equilibrium, the binding of the labelled 8-MOP was measured and the concentration of the free (labelled) drug was plotted against the increasing total concentration (labelled and unlabelled) of 8-MOP.

5-MOP or 8-MOP. The results of these experiments are presented in Table 1. The concentration increase of the drug within the epidermis was calculated for the original weight of the tissue sample and was taken as an indication and measure of the binding affinity. The concentration of 8-MOP was increased within the epidermis by ten to twenty fold over its concentration in the buffer, while 5-MOP appears to bind with a stronger affinity, resulting in a much higher concentration of the drug within the epidermis (250–500 fold increase). Removal of the horny layer by repeated stripping prior to the binding experiment had no effect on the extent of the binding of the two methoxypsoralens by the epidermis. No difference in the binding was observed between epidermis samples obtained by heating to 52°C and those obtained by stretching the skin. As was the case with binding to serum proteins, competition in binding to epidermis between 5-MOP and 8-MOP could not be detected and the binding of each appears to be independent of, and unaffected by, the presence of the other. In a similar manner, no binding competition was found between either one of the two photosensitizers and hydrocortisone, fluocinonide and acetyl salicylic acid.

DISCUSSION

The slow dialysis rate of 5-MOP and 8-MOP, as compared with the dialysis rate of other substances with a similar molecular weight, may be due to the high hydrophobicity and low water solubility of the two methoxypsoralens. The resulting long dialysis times required to reach equilibrium introduces the possibility of artifacts taking place, especially as regards changes in binding by serum proteins. Although the possibility of such changes in either binding affinity or binding capacity cannot be completely ruled out, dialysis experiments with substances requiring shorter dialysis times to reach equilibrium suggest that no such changes occurred under the experimental conditions employed here. Unfortunately, rapid methods for measuring dissociation constants (Colowick & Womack, 1969) are not applicable in this case due to the very slow dialysis rate of the two photosensitizers.

Under therapeutic conditions, when oral administration of 8-MOP is employed, serum concentrations of the latter are in the range of 100–1000 ng/ml. Under these conditions, about 75–80% of the drug will be reversibly bound to serum proteins and only 20–25% of the total concentration represents the effective (or bioavailable) drug concentration, which is free to diffuse and equilibrate with the various skin layers.

The constant binding of 98–99% of 5-MOP to serum proteins, found over the concentration range of 5 ng to 230 μg/ml, indicates a high affinity of binding with a dissociation constant apparently lower than 10^{-6} M or a higher number of binding sites per protein molecule. To measure such binding, 5-MOP concentrations above 10^{-3} M (216 μg/ml) are required. Due to the poor solubility of 5-MOP in aqueous solutions, such high concentrations could not be reached and the dissociation constant could not be determined.

There is no doubt that heating the skin to 52°C introduces some changes in the epidermis which, among others, allow its easy separation from the corium. Comparing the binding of the two photosensitizers, (Table 1) however, suggests that these changes do not alter the binding characteristics of the two drugs and heat separated epidermis can be employed for such studies, at least with these two substances.

The similar binding affinities to serum proteins and to albumin suggests that the binding in serum takes place mainly to the albumin fraction. Binding to other protein fractions has not been measured directly and cannot be ruled out; however, since the binding capacity of serum proteins for 8-MOP can be accounted for by the albumin present, it is likely that the binding takes place mainly to serum albumin.

The ability of the epidermis to bind and to concentrate the photosensitizers suggests that, under conditions of oral therapy, the actual drug concentration in the epidermis may be higher than the measured serum levels. This ability of the epidermis to concentrate the two psoralen derivatives is a property of the epidermal tissue only and is not influenced by prior removal of the horny layer.

The large difference in binding affinity and the apparent lack of any cross interaction of the two photosensitizers in binding to serum proteins and to epidermal structures are difficult to explain in view of the similarity in structure. Although the binding to serum proteins is not directly related to the photosensitizing action of the methoxypsoralens, the lack of cross interaction in binding to the epidermis suggests that these two photosensitizers bind to different epidermal sites and may induce photosensitivity in different ways—by interacting with different cellular structures under the influence of light. Although the binding measured here is a reversible binding and not induced by UV-A irradiation, it is likely that the photosensitizer will interact, under the influence of light, with these structures to which it is bound and on which it is concentrated as a result of this binding.

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Inhibition of human blood platelet aggregation by photochemotherapy *in vitro* and *in vivo*[*]

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SUMMARY

Irradiation of human blood platelets with UV-A *in vitro* impairs their ability to aggregate after challenge with collagen. There also appears to be mild and transient inhibition of platelet aggregability in psoriatics during the fourth week of photochemotherapy.

Photochemotherapy involves the administration of the photosensitizing drug 8-methoxypsoralen (8-MOP) followed by irradiation of the skin with longwave ultraviolet radiation (UV-A) in doses ranging from 1 to 30 J/cm^2 at frequent intervals for an indefinite period. Up to 40% of this radiation may penetrate into the dermis (Everett *et al.*, 1966), and since it is estimated that the total blood volume, depending on the thermoregulatory state, circulates through normal skin approximately every 2–7 min (Shuster & Marks, 1970) the effect of UV-A on the formed elements of the blood needs to be evaluated. So far, most attention has been directed towards nucleated cells, especially lymphocytes (Cormane *et al.*, 1977; Mourelatos *et al.*, 1977; Morison, Parrish & Bloch, 1978). In this study we report a preliminary investigation into platelet function under simulated and actual conditions of photochemotherapy.

MATERIALS AND METHODS

Platelet aggregation tests

Platelet-rich plasma was prepared from citrated whole blood by centrifugation in polypropylene tubes at 150 *g* for 15 min at room temperature. The platelet-rich supernatant was collected, a platelet count performed, and then platelet-free plasma was obtained from the same sample by further centrifugation of the residue for 20 min at 1500 *g*. Platelet aggregation was induced in stirred 0·5 ml aliquots of platelet-rich plasma at 37°C by the addition of 1 μg collagen suspended in 10 μl buffered solution at pH 2·7 (Collagenreagent Horm, Hormon-Chemie). The tests were carried out in siliconized glass cuvettes in a photoelectric titrator (EEL Titrator, Evans Electroselenium Ltd) connected to a potentio-

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[*] A brief report of these findings was presented at the Winter Scientific Meeting of the British Association of Dermatologists (Investigative Group, B.A.D.), 1979.

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metric recorder (Servoscribe RE511, Smiths Industries Ltd) calibrated so that light transmission through the platelet-rich plasma produced no deflection, and light transmission through the platelet-free plasma produced a full-scale deflection. Per cent platelet aggregation was measured as a change in light transmission of the platelet-rich plasma 6 min after addition of collagen.

In vitro experiments

Platelet-rich plasma was obtained from four healthy adults, four adults with extensive plaque psoriasis, and from four adults with palmoplantar pustular psoriasis 2 h after ingestion of approximately 0·5 mg of 8-MOP per kg of body weight. The mean plasma level of 8-MOP in the latter as determined by high pressure liquid chromatography (Hensby *et al.*, 1978) was 125·9 ± 66·4 ng/ml. One portion of each sample was exposed continuously in a translucent polypropylene test tube at ambient temperature to 4·8 mW/cm^2 of UV-A (320–400 nm, maximum at 365 nm) from PUVA 180 and 200 radiation units (H.Waldmann GmbH & Co., Germany); aliquots were withdrawn at 30 min intervals for platelet aggregation tests. Controls included aliquots of platelet-rich plasma treated in an identical way but screened from UV-A by tinfoil, and samples tested immediately after harvesting.

In vivo experiments

Whole blood platelet counts, platelet counts in platelet-rich plasma, and collagen-induced platelet aggregation tests were performed on blood samples from eight adults with widespread plaque psoriasis who were receiving photochemotherapy with oral 8-MOP and whole body UV-A in a PUVA 6000 radiation unit (H.Waldmann GmbH & Co., Germany) whose spectral output is identical to the radiation sources used in the *in vitro* experiments. The tests were performed in triplicate before the start of therapy and at mean intervals of 8 days during the course of treatment which was administered according to the schedule advocated by Wolff *et al.* (1977). On each occasion the blood sample was withdrawn at the end of a UV-A exposure, approximately 2½ h after ingestion of 8-MOP.

For controls, identical tests were carried out on blood samples from eight adults with widespread plaque psoriasis during inpatient treatment with the 'Ingram regimen' of dithranol, tar baths, and UV-B (Ingram, 1953). The tests were performed before the start of therapy and at weekly intervals thereafter.

RESULTS

In vitro experiments

The results of the *in vitro* experiments are given in Fig. 1a–c. The irradiated platelets showed a loss of their ability to aggregate compared with the unirradiated samples after 120 min of exposure (cumulative UV-A dose 35 J/cm^2), and this reached statistical significance at 150 min (mean per cent reduction of aggregation 72 ± 7 s.e. mean; $P<0·01$). The inhibition of collagen-induced platelet aggregation was independent of the presence of 8-MOP in the plasma, and of the presence of psoriasis in the blood donor. One sample from a healthy adult failed to aggregate at the end of 3 h even though it was kept in the dark, which accounts for the low mean percent aggregation of the unirradiated samples at this point in this experiment (Fig. 1c). This may have been due to the unrecognized ingestion of an aspirin-like compound.

In vivo experiments

Neither the whole blood platelet count nor the platelet count in the platelet-rich plasma altered significantly during the course of dithranol treatment and photochemotherapy. The results of collagen-induced platelet aggregation tests are given in Fig. 2a and b. Platelet aggregation remained unchanged

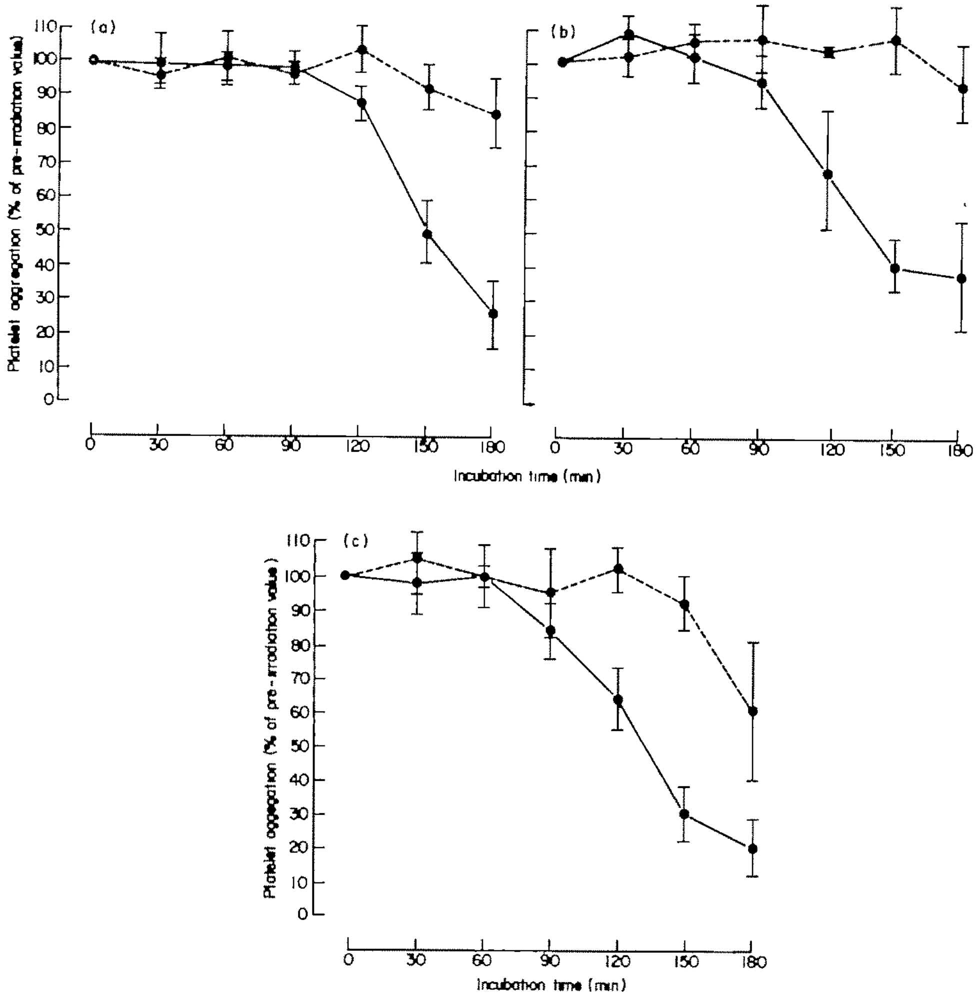

FIGURE 1. Inhibition of collagen-induced platelet aggregation by irradiation of platelet-rich plasma *in vitro* with UV-A. (Solid line: irradiated samples; broken line: samples kept in the dark. Mean ± s.e. mean; $n = 4$; $P < 0{\cdot}01$ at 150 min). (a) Samples from psoriatics (8-MOP present); (b) samples from psoriatics (no 8-MOP); (c) samples from healthy adults (no 8-MOP).

during treatment with dithranol (Fig. 2a). However, in the group of psoriatics treated with photochemotherapy (Fig. 2b) the mean per cent aggregation had fallen after 16 days of treatment, and at the end of 24 days (mean cumulative UV-A dose 35 J/cm^2) the reduction in mean platelet aggregation reached statistical significance (mean per cent reduction of aggregation 20 ± 11 s.e. mean; $P < 0{\cdot}01$). Despite continuation of photochemotherapy for a further 8 days, restoration of platelet aggregation to pretreatment values took place. This may have been related to the development of pigmentation, and to the reduction in frequency of photochemotherapy at this stage in some patients due to clearing of the rash.

DISCUSSION

These experiments indicate that irradiation of human blood platelets with UV-A *in vitro* impairs their

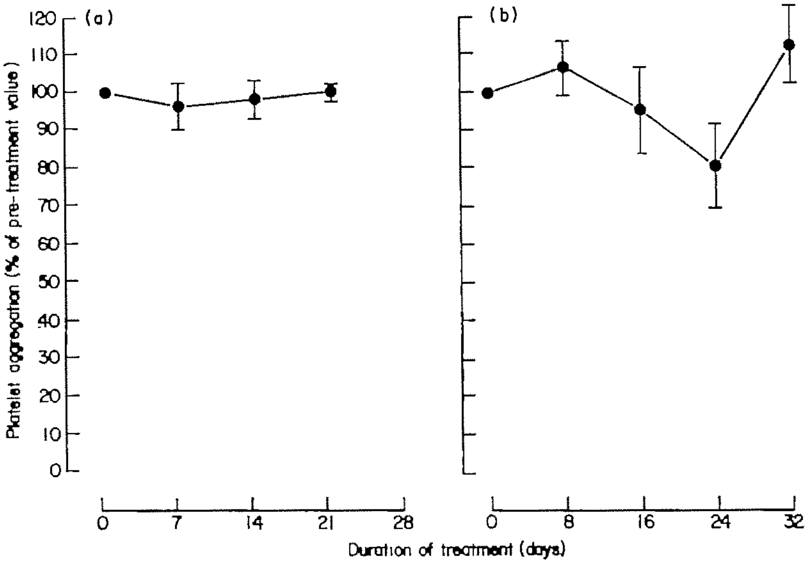

FIGURE 2. Inhibition of collagen-induced platelet aggregation during photochemotherapy. (a) Aggregation during dithranol treatment (Mean ± s.e. mean; n = 24); (b) aggregation during photochemotherapy (Mean ± s.e. mean; n = 24; $P < 0{\cdot}01$ at 24 days).

ability to aggregate after challenge with collagen. There also appears to be mild and transient inhibition of platelet aggregability in psoriatics during the fourth week of photochemotherapy.

Aggregation of platelets by collagen in low concentration, such as was employed in our experiments, involves activation of the prostaglandin biosynthesis pathway. Collagen activates phospholipase A_2 which catalyses the release of arachidonic acid from platelet phospholipids. In platelets the biologically active prostaglandins are prostaglandin G_2, prostaglandin H_2 and thromboxane A_2 (Hamberg *et al.*, 1974; Hamberg, Svensson & Samuelsson, 1975). These short-lived intermediates of the cyclo-oxygenase pathway are themselves aggregating, and also cause the release of endogenous ADP from the platelet granules which enhances further aggregation. In addition, endothelial cells can convert the endoperoxides released by stimulated platelets into prostacycline which inhibits platelet aggregation (Moncada *et al.*, 1976) and thereby plays an important role in the regulation of platelet aggregation *in vivo*. The ways in which UV-A might interact with this pathway are uncertain, but possibly of some importance. Although the ability of UV-A to inhibit aggregation of platelets is probably not of clinical significance, it may provide a valuable approach to the analysis of the molecular basis of platelet aggregation, and of the possible effect of UV-A on prostaglandin synthesis.

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Bowenoid lesions, Bowen's disease and keratoacanthomas in long-term PUVA-treated patients

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SUMMARY

Four patients are described, two with histologically proven multiple foci of bowenoid lesions, one patient with bowenoid lesions and Bowen's disease, and one patient with two keratoacanthomas, most of the lesions occurring in non sun-exposed but photochemically treated areas of the body. These patients had received long-term PUVA treatment for their psoriasis. Two patients had a history of arsenic intake. The possible relationship of these epidermal lesions to light treatment is discussed.

Oral 8-methoxypsoralen photochemotherapy (PUVA) has been a widely accepted modality for the successful treatment of widespread therapy-resistant psoriasis. PUVA treatment has been in operation since 1974. Numerous clinical reports attest its efficacy (Parrish *et al.*, 1974; Weber, 1974; Wolff, Hönigsmann & Gschnait, 1975; Wolff *et al.*, 1976; Hofmann, Plewig & Braun-Falco, 1976a, b; Melski *et al.*, 1977; Braun-Falco, Hofmann & Plewig, 1978).

We have had experience with this kind of therapy for about 4 years. The question of long-term side-effects is still open. The main concern centres around premalignant lesions or frank malignancies following long-term PUVA therapy with high cumulative UV-A doses.

In an earlier report (Braun-Falco, Hofmann & Plewig, 1977) we noticed bowenoid lesions in the epidermis of the uninvolved skin in a 50-year-old male psoriatic patient after forty PUVA treatments. He had received arsenic medication 15 years previously, but there were no cutaneous signs of arsenical keratoses or tumours.

In the meantime we have the impression that long-term PUVA treatment may be able to induce cutaneous malignancies. We therefore wish to report three further patients who developed histologically premalignant skin lesions. The four patients were taken from a group of fifty-four patients

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who attended for PUVA therapy at regular intervals for between 1 and 3 years. A total of seventy-two biopsies were secured from these fifty-four patients. The mean number of PUVA treatments before biopsies was ninety-five ± forty-two, with a maximum of 261 treatments. In detail forty-four patients received up to 100 treatments, twenty-five patients between 100 and 200 treatments, and two patients over 300 treatments. The cumulative UV-A dose was 658 ± 519 J/cm^2. Ten patients had received more than 1000 J/cm^2, one patient more than 2000 J/cm^2, and one patient 3058 J/cm^2.

CASE REPORTS

Case 1

Patient S.K., aged 50 years, farmer, skin type III.

History. Since 1948 he has had psoriasis vulgaris of the chronic-stationary type, and was hospitalized for psoriasis in 1950. He has never had systemic therapy for psoriasis; no glucocorticosteroids, methotrexate or arsenic. His mother had psoriasis.

PUVA therapy. Standardized PUVA conditions were used with 40 mg 8-MOP tablets (Meladinine, Basotherm, Germany) and irradiation 2 h later in a high intensity lie-down box (Protocol of the European Cooperative Clinical Trial, 1975; Hofmann *et al*., 1976a). The initial treatment phase was followed by maintenance therapy at weekly intervals until May 1978. At this time the patient had received 114 PUVA treatments and a cumulative UV-A dose of 747 J/cm^2.

Laboratory data were all within normal limits.

Biopsies. Four biopsies were secured, always from non sun-exposed but PUVA-treated lesion-free skin of the buttocks. There were no clinical signs of light-induced skin changes, except for pigmentation. The biopsies were routinely processed for light microscopy and stained with haematoxylin-eosin, Giemsa, PAS, Hale-PAS, and Elastica.

The first biopsy on 16 June 1976 after fifty-six PUVA treatments with a total UV-A dose of 365 J/cm^2 showed a normal epidermis. Numerous clear cells (melanocytes) at the dermo-epidermal junction were seen (Braun-Falco *et al*., 1977). There were increased melanin granules in the basal cell region. No atypical cells. Normal appearing elastic fibres with questionable rarification of the subepidermal elastica plexus.

Diagnosis: normal appearing skin; PUVA-induced clear cell activity and pigmentation.

A second biopsy on 26 July 1977 after ninety-one PUVA treatments, total UV-A dose of 619 J/cm^2, showed the following: partly irregular configuration of epidermis with loss of the dermal papillary pattern. Several foci of atypical cells with large, vacuolated and prominent polymorphic nuclei. The orderly arrangement of cells in the lower and middle parts of the Malpighian layers was disturbed. Frequent atypical mitoses. Slightly increased number of clear cells at the dermo-epidermal junction with increased pigment within basal and suprabasal cells. No signs of actinic elastosis (Fig. 1).

Diagnosis: bowenoid lesion; partly atrophic epidermis; PUVA-induced clear cell activity and pigmentation.

A third biopsy on 5 January 1978 after 102 PUVA treatments, total UV-A dose of 695 J/cm^2, showed the following: prominent basket-weave-like orthohyperkeratosis. Disturbance of the normal stratification of the epidermis. Numerous atypical cells.

Diagnosis: bowenoid lesion; PUVA-induced orthohyperkeratosis.

The fourth biopsy was on 18 July 1978 after 114 PUVA treatments, total UV-A dose of 747 J/cm^2.

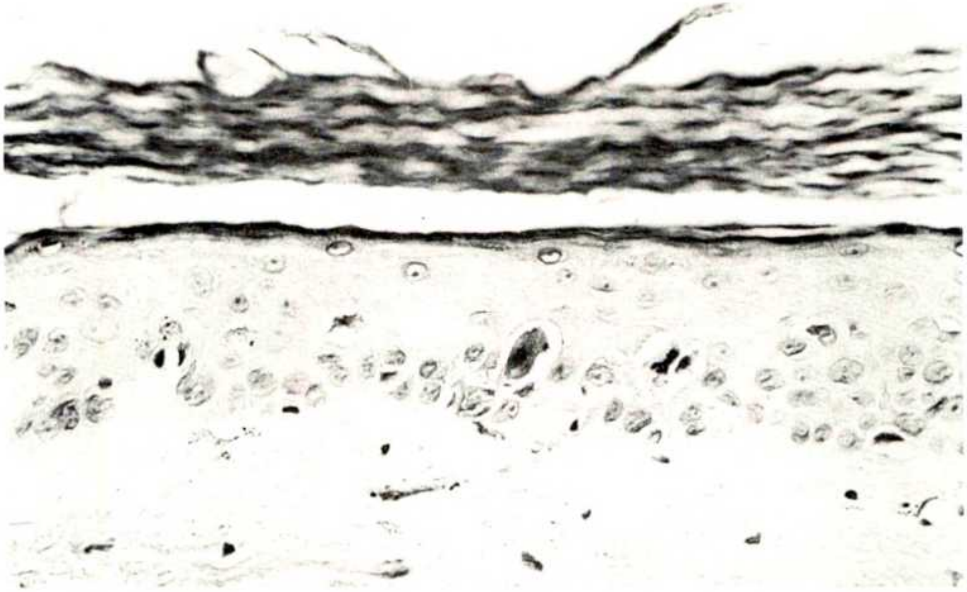

FIGURE 1. Bowenoid lesion. Clinically normal looking skin of the buttocks in a 50-year-old male patient after ninety-one PUVA treatments (total UV-A dose of 619 J/cm²). Foci of large vacuolated cells with prominent and polymorphic nuclei. Frequent mitoses (H & E, × 260).

There had been an interval without PUVA treatment of 2 months. It showed the following: normal appearing epidermis, no atypical cells. Increased pigmentation in the basal cell region, pigment deposition in macrophages. Loss of elastic fibres in the subepidermal plexus.

Diagnosis: normal appearing skin; PUVA-induced pigmentation.

Case 2

Patient T.R., aged 39 years, businessman, skin type III.

History. Psoriasis vulgaris since 1958 for which he has twice been hospitalized. Treatment with systemic corticosteroids for several months, no methotrexate, but arsenic intake in 1959 and 1960 of unknown dose. No family history of psoriasis.

PUVA therapy. Under the same conditions as in Case 1. PUVA therapy was performed up to October 1978 with 261 PUVA treatments, total UV-A dose of 3056 J/cm².

Laboratory data were all within normal limits.

Biopsies. Two biopsies were secured as in Case 1 and routinely processed as described above.

The first biopsy on 25 July 1977 after 176 PUVA treatments, total UV-A dose of 1575 J/cm², showed: prominent orthohyperkeratosis ('Lichtschwiele') (Miescher, 1940). Numerous clear cells in the basal cell layer with an increased melanin content. In the interfollicular epidermis and in the acroinfundibula there is focal derangement of atypical cells with condensed nuclei, vacuolated cytoplasm, polymorphic nuclei, and numerous prominent nucleoli. There are numerous mitoses (Fig. 2).

Diagnosis: multifocal bowenoid alterations; PUVA-induced clear cell activity and melanization.

A second biopsy on 5 October 1978 after 261 PUVA treatments, total UV-A dose of 3056 J/cm^2 showed: slightly atrophic epidermis with loss of the dermal papillary pattern. Many clear cells in the basal cell region with increased pigmentation. Incontinence of pigment. No atypical cells.

Diagnosis: atrophic epidermis; no cell atypia; PUVA induced pigmentation.

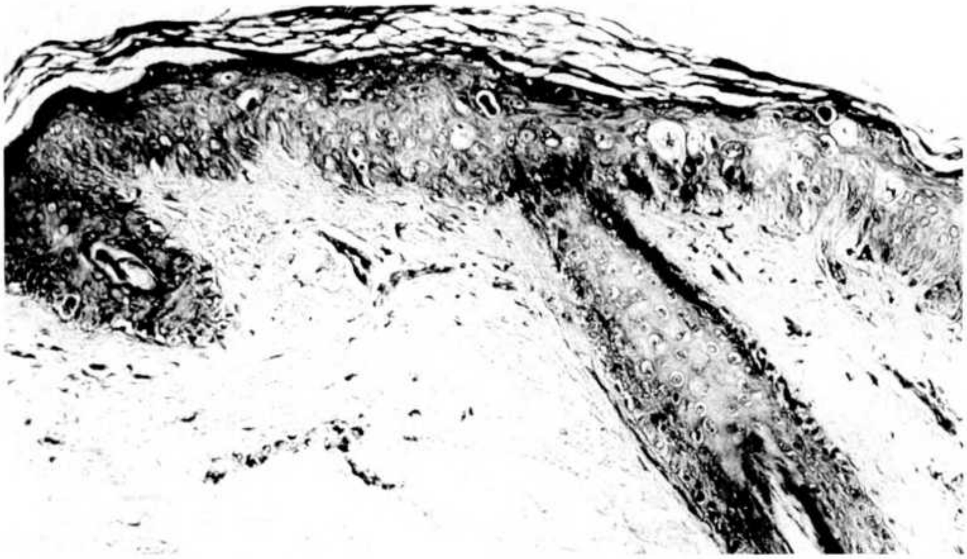

FIGURE 2. Bowenoid lesions. Clinically normal looking skin of the buttocks in a 39-year-old male patient after 176 PUVA treatments (total UV-A dose of 1575 J/cm^2). In the interfollicular epidermis and in the acroinfundibula there is focal derangement of atypical cells with vacuolated cytoplasm, polymorphic nuclei and numerous mitoses (H & E, × 110).

Case 3

Patient F.L., aged 51 years, civil servant, skin type III.

History. Severe psoriasis vulgaris of the exanthematic-guttate type since 1958; twice hospitalized for psoriasis in 1975. Arsenic intake in 1960 and 1961. No methotrexate, no ionizing radiation. Diabetes mellitus being treated with insulin. His brother had psoriasis.

PUVA therapy. Under the same conditions as in Cases 1 and 2, lasting until the writing of this report. Laboratory data were all within normal limits.

Biopsies. Three biopsies were secured and processed as in Cases 1 and 2.

The first biopsy on 27 April 1976 after forty PUVA treatments, total UV-A dose of 219 J/cm^2, showed: irregular stratification of the epidermis with numerous atypical cells with polymorphic nuclei, numerous prominent nucleoli, multiple mitoses in the basal and suprabasal cell layers. Increased number of clear cells at the dermo-epidermal junction.

Diagnosis: bowenoid lesion; PUVA-induced clear cell activity. The patient and the findings of this biopsy including microphotographs were reported in an earlier study (Braun-Falco *et al*., 1977).

The follow-up of this patient revealed the following new data:

A second biopsy (1 June 1977) after ninety-one PUVA treatments, total UV-A dose of 707 J/cm^2

and a third biopsy (1 August 1978) after 142 PUVA treatments, total UV-A dose of 1047 J/cm^2, showed: normal epidermis; no atypical cells; PUVA-induced clear cell activity.

Follow-up. During the summer of 1978 there was slow development of a slightly erythematous and elevated plaque on the proximal part of the left thumb, unresponsive to PUVA therapy. Clinical diagnosis: Bowen's disease.

A biopsy was taken from this lesion on 6 December 1978. At this time the patient had received 150 PUVA treatments, total UV-A dose of 1166 J/cm^2. The biopsy revealed: loss of rete ridges, slightly acanthotic epidermis. Cellular dysplasia throughout the stratum Malpighii; dyskeratotic cells. Numerous mitoses, basilar and suprabasilar. Focal loss of the granular layer, ortho- and parakeratosis (Fig. 3). Histological diagnosis: Bowen's disease.

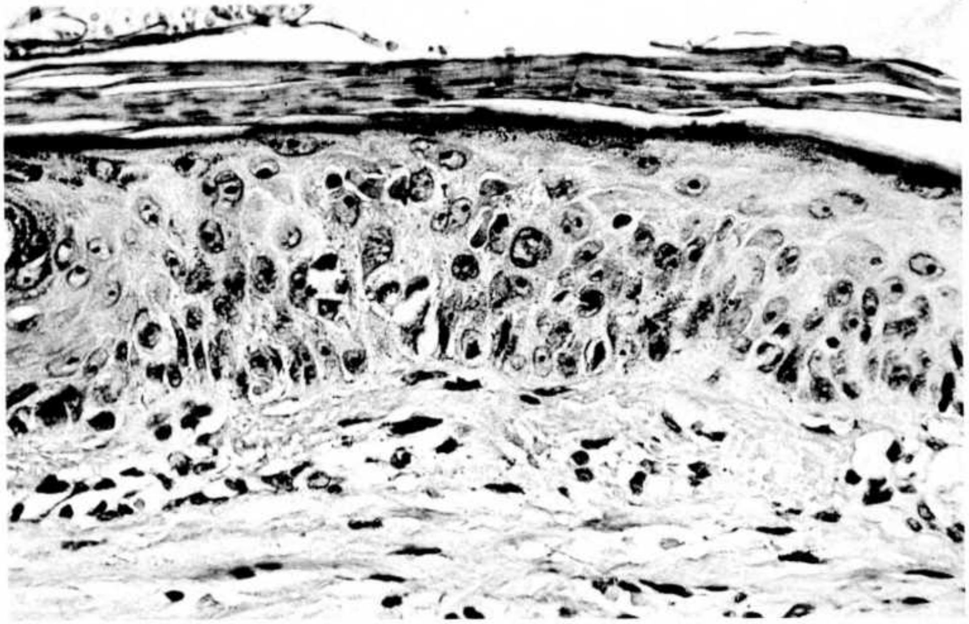

FIGURE 3. Bowen's disease on the proximal part of the left thumb in a 51-year-old male patient after 150 PUVA treatments (total UV-A dose of 1166 J/cm^2). Cellular dysplasia throughout the stratum Malpighii with many dyskeratotic cells. Numerous mitoses. Focal loss of the granular layer (H & E, ×260).

Case 4

Patient H.S., aged 69 years, truck-driver, skin type III.

History. Since 1922 psoriasis vulgaris of the chronic-stationary type. No systemic treatment with steroids, methotrexate or arsenic. No family history of psoriasis.

PUVA therapy. Under the same conditions as in Cases 1–3, lasting until the writing of this paper. Laboratory data were all within normal limits.

Follow-up. In May 1978 simultaneous appearance of two enlarging dome-shaped reddish-brown nodules with a central keratotic plaque on the left lower leg. The clinical diagnosis was keratoacanthoma.

Biopsies. Excisional biopsies of the tumours were taken on 1 and 23 June 1978 after 108 and 111 PUVA treatments respectively and a total UV-A dose of 763 and 786 J/cm^2 respectively. They revealed prominent and irregular acanthosis and papillomatosis with lateral epithelial lip formation. In the centre of the lesion there was a large, irregularly shaped crater filled with keratin. In the strands of epidermis protruding into the dermis were several cells of atypical appearance. Slight perilesional inflammatory cell infiltrate in the dermis (Fig. 4).

Diagnosis: keratoacanthomas.

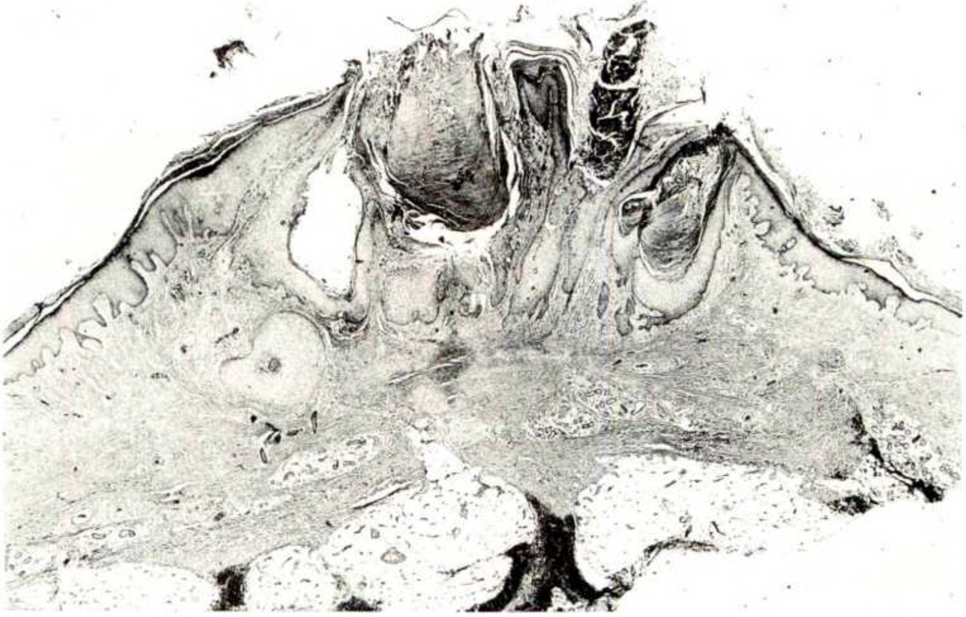

FIGURE 4. Keratoacanthoma on the left lower leg in a 69-year-old male patient after 111 PUVA treatments (total UV-A dose of 786 J/cm^2). Irregular acanthosis and papillomatosis with epithelial lip formation. In the centre of the lesion there is a large, irregularly shaped crater filled with keratin. Perilesional inflammatory cell infiltrate (H & E, ×45).

DISCUSSION

At present it is still debatable whether or how often PUVA-induced premalignancies or malignant tumours of the skin occur, especially in patients undergoing long-term high intensity treatment. We here present four patients in whom such skin lesions are at least open for discussion.

The first patient had never had arsenic, a well known tumour inducing agent (Knoth, 1966; Jung & Trachsel, 1970; Bettley & O'Shea, 1975). The latency period for the appearance of malignancies after arsenic is usually many years, and may be as long as 20–35 years (Ehlers, 1968). In two of the four biopsies definite bowenoid atypia was seen, although there were no clinical lesions. Also there were no palmar pits.

In the second patient there was a history of arsenic intake. Clinically there were no arsenical keratoses or palmar pits. In one of the two biopsies histologically there were definite multifocal bowenoid lesions.

As mentioned in the introduction, we noticed bowenoid lesions in one of the earliest PUVA-treated patients in our series. This is Case 3 in the present report. Data up to 1976 and histopathological photographs of this patient who also had taken arsenic, were published elsewhere (Braun-Falco *et al.*, 1977). The latest biopsy from this patient now reveals clinically and histologically Bowen's disease.

The fourth patient, who has never had arsenic, developed two keratoacanthomas on non sun-exposed, but PUVA-treated parts of the left lower leg. Keratoacanthomas may be multiple (Bönninger & Burg, 1979) and may occur in non sun-exposed sites although the majority of these tumours are directly related to the amount of sunlight exposure (Reed, 1972; Gründer & Hundeiker, 1973).

It is impossible at the present time to separate the effect of the tumour-promoting arsenic from that of PUVA. It is conceivable however that arsenic and PUVA may be acting synergistically. Close observation especially of patients with a previous history of arsenic intake is mandatory.

We are not aware of any written account of premalignancies or tumour formation in PUVA-treated patients (Bergfeld, 1977; Heidbreder, 1978), although this was reported orally at the American Academy Meeting in San Francisco in December 1978.

Detailed clinical studies of over 150 patients who are receiving high intensity PUVA treatment for up to 3½ years and also detailed histopathological studies of seventy-two biopsies from fifty-four patients with special emphasis on epidermal and dermal components will soon be published elsewhere.

ACKNOWLEDGMENTS

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ADDENDUM

At the time this paper was reviewed, a report appeared which described Bowen's disease and squamous cell carcinoma in a 32-year-old PUVA-treated patient (Tam, van Scott & Urbach, 1979).

British Journal of Dermatology (1979) **101**, 693.

The removal of amateur tattoos by salabrasion

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SUMMARY

Due to the recent upsurge in self-inflicted tattoos in teenagers it was decided to employ for their removal the method of salabrasion, i.e. abrasion with table salt. This technique was recently used by Manchester (1973, 1974) for the removal of commercial tattoos.

Fourteen patients with twenty-eight tattoos were treated; ten tattoos showed a good response, i.e. complete removal of the tattoo or only tiny flecks of pigment remaining visible; eleven showed a fair response, i.e. the tattoo, although much lighter in colour, was still legible but the patient was satisfied with the result; three showed a poor response, i.e. the tattoo appeared as if untreated; four (two patients) defaulted.

In general, as could be expected, the darker the tattoo the more difficult was its removal and those on fingers were particularly resistant.

For centuries the removal of tattoos has been a frustrating problem; the multiplicity of methods which have been employed verifies that none is wholly satisfactory. Most techniques employ mechanical, chemical or surgical means or combinations thereof. During the reign of the Emperor Claudius garlic, excrement of pigeons, decomposed urine, pepper and lime, mother's milk, strong vinegar, sulphuric acid and cantharides were used; agents producing superficial burns such as phenol, nitric acid and carbon dioxide snow have also been popular (Shie, 1928). Tattoo artists cover the area with tannic acid paste and tattoo this into the design (Variot, 1888). Methods employed at present by the medical world include surgical removal, either shaving, simple excision or excision and skin grafting, scalpel cross-hatching, skin coagulation by electrical current or chemicals, dermabrasion and cryosurgery, and even the laser beam has been tried (Goldman, Hornby & Meyer, 1965; Goldman *et al.*, 1967; Yules *et al.*, 1967). In all methods the cosmetic results are often unsatisfactory. Abrasion with salt was first described by the Greek physician Aetius in 543 A.D.; the method was used in this century by Klövekorn (1935) and Crittenden (1971), the latter coining the name 'salabrasion'. Manchester (1973, 1974) reported on the use of table salt in the removal of commercial tattoos.

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PATIENTS AND METHODS

Fourteen patients, eleven females and three males ranging in age from 14 to 23 years were included in this trial. They had a total of twenty-eight amateur Indian ink tattoos on the upper limbs.

The area was first cleaned with isopropyl alcohol and local anaesthesia was produced by injecting 1% plain lignocaine; vasoconstrictors were not used as they cause difficulty when ascertaining the end point of the procedure. A sterile gauze swab was slightly dampened in cold water and then dipped in table salt. The affected area was rapidly abraded with the salt impregnated swab. After several minutes erythema developed and soon the epidermis was rubbed away. The swab and salt were changed whenever the swab became sodden and the salt was losing its abrasive effect. The treatment was continued until the area was a deep red colour but frank bleeding had not occurred.

A dry sterile swab was then applied under a bandage and the patient told to change this daily. A 10-day course of oxytetracycline 250 mg, four times daily, was prescribed. On changing the dressing pigment could be seen appearing in the swab and in a few days the area began to heal. After 2 weeks a crust had formed and 4–5 weeks later this separated, leaving a well healed area.

RESULTS

Of the twenty-eight tattoos treated ten showed a good response, i.e. the tattoo was completely removed or only tiny flecks of pigment were faintly visible (Fig. 1); eleven showed a fair response, i.e. the tattoo, although very much lighter in colour, was still just visible but the patient was satisfied with the result; three showed a poor response, i.e. the tattoo remained as if untreated and four (two patients) defaulted.

Of the good responses the vast majority were salabraded on one occasion only. A few of those in the fair range required more than one treatment. Two of the three poor results were on fingers. In general, the darker the tattoo the more difficult was its removal and those on fingers were particularly resistant. Some of the areas healed with a degree of hypopigmentation and prominence of the pilosebaceous follicles. One area on a finger became superficially infected. None showed obvious scarring, hyperpigmentation or keloid formation as described by Manchester (1974).

DISCUSSION

Long waiting lists for the removal of tattoos have always posed a problem and the recent craze for self-inflicted Indian ink tattoos in adolescents has served to accentuate it. When the tattoo (usually a boy/girlfriend's initials, a name, a historic date—'1690', 'love' or 'hate') is needled in, it is in the mistaken belief that it can be deleted readily by sour milk, vinegar, or as in one case, lavatory cleanser. On finding this is not so, or when the boy/girlfriend changes, a desperate search for a method of removal begins.

Before embarking on salabrasion we considered it worthwhile spending time psychologically preparing the patient: the various available methods of treatment and their advantages and disadvantages were discussed. The technique of salabrasion was then described but the patients were warned that it would possibly not remove the pigment completely and even if it did the skin would most probably be different in texture from the surrounding skin. However, they were assured that if it proved unsatisfactory then the other methods would still be available to them. Those whose tattoos would normally have required excision and grafting were pleased that they would have only one blemish.

The method is extremely simple, and indeed an occasional patient would admit to having tried it later at home on another tattoo. Unfortunately the pain produced by rubbing salt on an unanaesthetized wound usually brings the exercise to an abrupt halt. Dry sterile gauze swabs were used as the

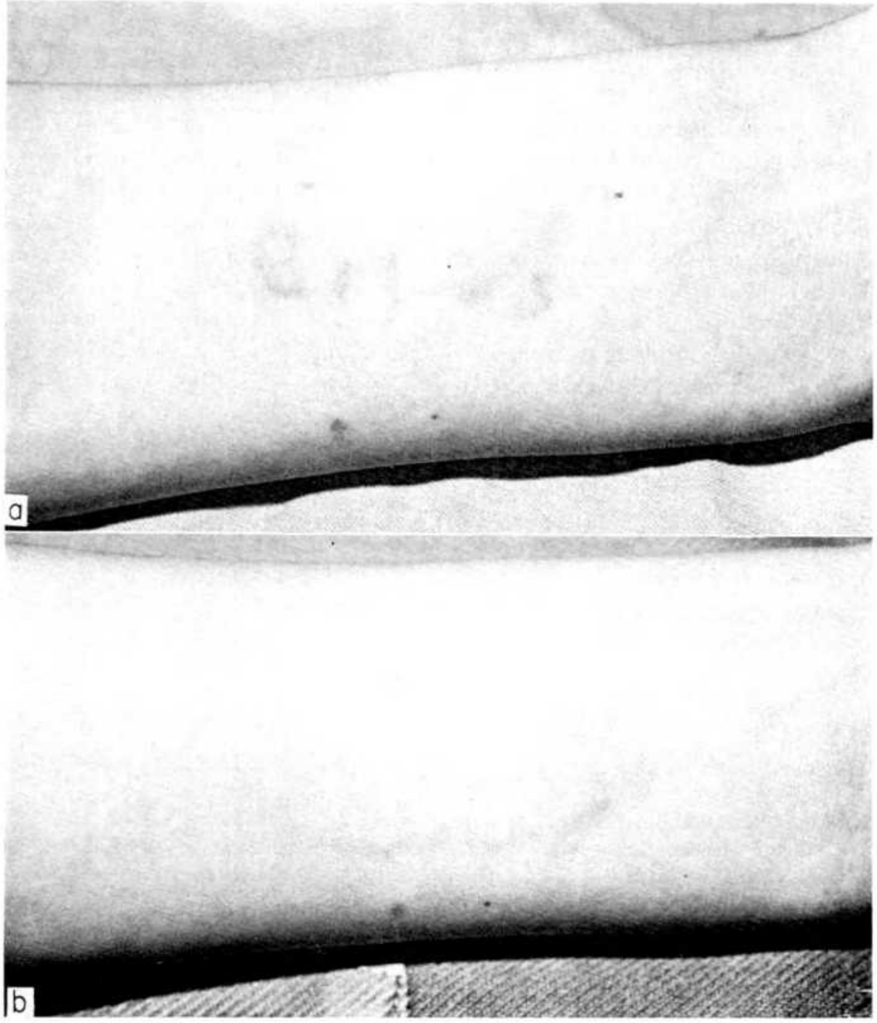

FIGURE 1. (a) Before treatment; (b) 10 months after treatment.

dressing; these became adherent to the skin and the patient was instructed to remove them daily. Although this could be painful they complied as they could see the ink appearing in the swab.

Manchester's (1974) results are somewhat similar to ours; he treated thirty-seven tattoos, six with poor results, eleven fair (undefined), ten good (undefined) and ten excellent, i.e. 'total disappearance of the tattoo with no apparent evidence of the treatment remaining'. None of our patients fell into this category.

The reason why abrasion with salt should be successful is still unclear. Shie (1928), Boo-Chai (1963), Thomson, Birdsell & Freiberg (1966) and Rook & Thomas (1952) have suggested that an inflammatory reaction causes mobilization of pigment which is then carried away via the lymphatic system. Clabaugh (1968) felt that this mechanism possibly plays a role in the natural fading of tattoos but probably only partially explains the disappearance of pigment after a tattoo has been abraded. Using dermabrasion he demonstrated clinically that the pigment migrated from the denuded dermal surface to form an outline of the tattoo on the dressing. Our patients confirmed this. He microscopically examined the exudate and found many macrophages with pigment granules in their cytoplasm. His conclusion was that the trauma of abrasion provokes an inflammatory reaction which enhances phagocytosis of free pigment and these pigment laden macrophages then migrate to the wound surface. Larnder & Singh (1974) suggested that salt, being hypertonic, may cause crenation of pigment-laden macrophages and thus free the pigment which can then be rephagocytosed and carried to the wound surface.

Koerber & Price (1978) salabraded a variety of commercial and amateur tattoos, leaving the salt on for periods of 0–24 h. They found that the best results were obtained by removing the salt immediately after salabrasion and that continued contact caused more scarring and hypopigmentation.

This series shows that salabrasion can be a useful alternative to the other methods of tattoo removal which all have basic disadvantages. Dermabrasion or shaving with a skin graft knife to a sufficient depth often produces hypertrophic scarring which takes a long time to resolve and eventually leaves pale scars. Excision or shaving and grafting result in a patch of skin which is paler than the normal surrounding skin and is also not hair bearing and this can be a problem on the dorsum of the forearm. The tannic acid tattooing method (Scutt, 1972) has in our hands produced unacceptable scarring. The laser can produce hypertrophic scarring and is not recommended for routine treatment.

The technique of salabrasion is extremely simple and can be practised by any medical practitioner. It is also very inexpensive in that it requires no technical equipment and for this reason could be undertaken, if desired, at a moment's notice.

We feel that in our practice we are now able to select the tattoos which are suitable for salabrasion: they should be relatively pale and should not be on the hand. If these criteria are fulfilled, this method can be ideal.

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Specific hyposensitization in atopic dermatitis

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SUMMARY

Specific hyposensitization is rarely advocated for the treatment of atopic dermatitis. Good results have been obtained in fifteen mainly adolescent and adult patients selected from over 3,000 patients with atopic dermatitis. Criteria included a history of exacerbations of the dermatitis after exposure to the antigen, an airborne antigen which could not be avoided, a rather distinctive clinical picture and symptoms bad enough to warrant the considerable difficulties involved.

The aetiology of atopic dermatitis is obscure. It is believed to be a multifactorial disease with a strong genetic predisposition and a variety of immunological and non-immunological abnormalities (Parish & Champion, 1973; Rajka, 1975; Champion & Parish, 1979). The association of atopic dermatitis with other undoubted allergic disorders such as hay fever, with positive immediate skin tests and with increased amounts of IgE, all indicate that immediate (anaphylactic) allergy plays an important role. However, eczema is not the hallmark of IgE-mediated reactions. Increased formation of IgG and depressed cell mediated immunity also occur (Rajka, 1975; Byrom & Timlin, 1979) and it has been postulated that the immunological abnormalities and clinical signs result from lack of control of B and T effector cells, possibly a defect of suppressor cells (Parish & Champion, 1973; Champion & Parish, 1979). Moreover, there are several other abnormalities in atopic dermatitis not attributable to allergy, e.g. the dry skin, the low itch threshold, the nervous influences, the tendency to vasoconstriction and pharmacological abnormalities.

In the management of most cases of established atopic dermatitis, allergen-avoidance based on positive skin immediate prick tests results in no improvement, and in doubts on the validity of the interpretation of the skin tests. However, recent studies indicate that cows' milk and other allergens can aggravate though probably do not initiate atopic dermatitis (Hammar, 1977; Atherton *et al.*, 1978) One of us (Di Prisco de Fuenmayor, 1979) confirmed that a high proportion (in this study 67%) of patients with atopic dermatitis (90% of those who also had respiratory disease) showed one or more positive skin tests to a battery of allergens. However, in only 31% were the prick test reactions considered relevant to the patients' symptoms, and in only 11% were they relevant to their dermatitis.

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Hyposensitization treatment is now firmly established as helpful in the treatment of hay fever, though the mode of action is unknown (Norman, 1978). Its value in the treatment of asthma is uncertain, though it is believed by some to bring about improvement in selected patients (*British Medical Journal*, 1978; Warner *et al.*, 1978).

Hyposensitization is rarely used in the management of atopic dermatitis, though it has been attempted in occasional cases over many years (Strauss & Kligman, 1957; Rajka, 1975), and in a small controlled trial (Kaufman & Roth, 1974). It has even been considered that atopic dermatitis is a contra-indication to hyposensitization treatment for other allergies.

Because of the paucity of information on the problem we here describe the results of hyposensitization treatment in fifteen carefully selected patients with atopic dermatitis.

CLINICAL MATERIAL

The fifteen patients were selected from over 3,000 cases of atopic dermatitis seen in 15 years, and were aged 6–14 years with a mean of 25. The criteria for attempting such treatment were:

(1) A history that exposure to the suspected allergen repeatedly caused itching (occasionally frank urticaria) and an exacerbation of their atopic dermatitis. Such exacerbations can last for minutes, hours or even a few days after a single exposure to an allergen, even if scratching is avoided
(2) A positive skin prick test, in a few cases confirmed by a RAST test.
(3) An inhalant allergen which could not readily be avoided. Injection therapy was undertaken only if reasonable attempts at allergen avoidance had failed. Food allergens were not included.
(4) Symptoms severe enough to warrant the difficulties and cost of treatment.
(5) All the patients with the first four essential criteria had a similar clinical picture. None had only the more usual prurigo-like picture with lichenification, or patches of true discoid eczema, although there are clinical and experimental precedents for believing that any type of eczema can be aggravated by IgE-mediated reactions (Jillson & Adami, 1955; Strauss & Kligman, 1957). All our patients did show the typical prurigo and lichenification but also showed rather diffuse ill-defined erythema, at times merging into atopic erythroderma—the sort of picture which might suggest an immediate weal type sensitivity. Most patients were very severely affected and had failed to respond to conventional therapy, including in-patient treatment, although oral anti-histamines were often of more value than in many cases of atopic dermatitis.

Hay fever and asthma provoked by the relevant allergen were absent in one-third of the cases, and were always mild and in themselves insufficient to warrant desensitization.

With some of the allergens it was not always possible to be sure that contact allergy might not be of some importance, as shown by Hjorth & Roed-Petersen (1976), but in the majority the history and clinical picture suggested systemic absorption following inhalation.

The most important allergen was, not surprisingly, house dust/house dust mite (nine cases); grass pollen was incriminated in four patients, and algae fulfilled our criteria in five patients (we now have altogether twelve patients who have allergies to these ubiquitous organisms (Champion, 1971)). Horse dander was included once. Usually only one allergen was included in the vaccines despite the fact that the patients often gave many positive skin tests and even a history that exposure to more than one allergen could cause itching. The dominant allergen as judged by the history was always chosen, rather than that giving the strongest positive skin test. Four patients were treated with more than one allergen.

The vaccines used were alum-precipitated pyridine extracted where available (Alavac, Bencard; Allpyral, Dome) but for the algae and lichens an aqueous vaccine (Bencard) was used. The course recommended by the manufacturers for the very sensitive patient was usually adopted, and often the dose increments had to be made more slowly than recommended. The injections were mostly given in the hospital out-patient department with all facilities available for treating a severe anaphylactic reaction. Seven of the patients had a single course of eight or more injections at weekly intervals, four patients had repeated courses, and four are being maintained on a long-term continuation course of monthly injections of the maximum dose given in the initial standard course.

RESULTS

Of the fifteen patients every one stated that they were at least a little better, no longer noticed the exacerbations produced by the allergen concerned and required less topical corticosteroid therapy. The improvement could be assessed as very good (estimated 80% better) in five, useful (50% better) in four, or marginal in six. No doubt some of the improvements in the last group were attributable to patients' desires to please their doctors, but even taking the first two groups alone there were nine who were unequivocally much better.

Improvement usually started after the fourth or fifth injection. It lasted in some cases for only a few weeks, usually some months and in a few cases a single course seemed to achieve an improvement maintained for years.

A gradual relapse prompted further courses in four patients. More recently we have tended to give continuation courses for a year or more.

PROBLEMS WITH TREATMENT

Several of the more severely affected patients had real problems during the course of their injections. Almost all noted an exacerbation of their dermatitis within a few hours, lasting for 2–3 days after each of the first injections; two patients noted quite severe generalized itching even after their prick-tests. Such reactions were considered a strong indication to continue treatment rather than abandon it and were treated for a short time with more intensive topical steroid therapy, and often oral antihistamines. Two patients required systemic corticosteroids to cover their reactions. No patient developed serious signs of generalized anaphylaxis.

DISCUSSION

It must be emphasized that this was a highly selected group of patients with difficult atopic dermatitis, many of whom had failed to respond to usual measures. These are the sort of patients in whom such treatment as long-term corticosteroids, immunosuppressives or PUVA therapy might have been considered. We certainly do not advocate hyposensitization treatment for the great majority of patients.

This was not a properly controlled trial. With such small numbers of carefully selected cases, and with such reactions following treatment, a double-blind trial would be almost impossible to achieve. We have seen well-marked improvement in a number of patients attending a clinic at weekly intervals for something as non-therapeutic as repeated pharmacological skin tests and who even asked for a further course. However, the improvement in nine of these fifteen patients was of a different order.

We believe that specific hyposensitization has a place in the occasional patient with severe atopic dermatitis but it is not a treatment to be considered lightly and can be expected to ameliorate only the allergic component of a multifactorial disease. Discussions with numerous colleagues have shown that it is a treatment very rarely undertaken in this country.

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Trachyonychia (rough nails)

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SUMMARY

The term trachyonychia is a useful one to describe roughening of the surface of many nails due to various causes. Psoriasis and alopecia areata are responsible for some cases. Lichen planus can roughen nails but the change is usually limited to a few nails and accompanied by discomfort. In many cases no cause can be found and these cases are somewhat arbitrarily listed either as twenty nail dystrophy of childhood or severe nail dystrophy.

The term trachyonychia was coined by Alkiewiez in 1950 to record cases of rough nails. He described three cases aged 19, 12 and 33 years respectively. The photographs illustrating his article are very similar to my own. Achten & Wanet-Ronard (1974) took up the term in a classification of nail dystrophies of known and unknown causes. They described seven cases: four were in one family, two in another and one independent; all but one was a child. Uniform pitting of nails has for a long time been known to be caused by psoriasis (Klingmuller & Reh, 1955) and by alopecia areata (Stuhmer, 1957). Uniform pitting will roughen the nail surface but does not produce as great a deformity as in the cases I am describing.

Hazelrigg, Duncan & Jarratt (1977) described a group of children in which excess ridging was the principal feature and named the condition 'the twenty nail dystrophy of childhood'. Following this article Baran & Dupre (1977) in a letter to the *Archives of Dermatology* pointed out that some cases are probably due to alopecia areata but where the whole nail is not involved lichen planus or psoriasis may be responsible. These observations were more fully expressed by Baran *et al.* (1978).

For many years now I have seen one or two cases each year of nail dystrophies in which roughening of the nail surface has been the presenting feature and affecting all or almost all finger and toe nails. The great majority of cases have been in children. These cases have proved to be very difficult to classify although some have been observed for a number of years. They may for convenience be placed in three groups: (a) alopecia areata or totalis; (b) twenty nail dystrophy of childhood; (c) 'severe' nail dystrophy. Clinically, however, there is very little difference between the nail changes in the three groups but for the time being groups (b) and (c) should be considered idiopathic.

The usual story is that the nails are normal at birth and then some time in the first decade some nails become roughened at the base and with continuing growth of the nails the whole nail plate becomes affected. Some cases start in the first year of life. Gradually, all or almost all nails become involved.

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The process often leads to the nail becoming thinner but occasionally thicker than normal. One or two nails often show koilonychia presumably because the nail becomes soft. In two cases actual loss of some nails occurred as a temporary phenomenon. Although it has not been possible to follow all cases, several have been observed for a few years and the impression has been that there is a gradual improvement in most areas. There is no clear cut distinction between groups (b) and (c) but they do seem to me to warrant separation until we have a better knowledge of the aetiology. Adult cases are put into the 'severe' nail dystrophy group. Childhood cases are somewhat arbitrarily divided between twenty nail dystrophy of childhood and severe nail dystrophy. The 'severe' cases include those in whom some nails are lost or colour changes and alterations in nail thickness are more apparent than is ridging. Ridging is the principal complaint in twenty nail dystrophy. A few case histories may serve to show the similarity and differences between the groups.

CASE HISTORIES

Alopecia areata

Case 1. H.B. aged 7. A young Arab boy with total alopecia and severe nail changes which appeared about 7 months before the hair fall. The nail changes are very similar to the idiopathic type to be described.

Case 2. P.W. aged 7, female. The nail disorder began 1 year previously and the mother described the changes as 'losing her nails'. She had had patches of hair fall on and off for 3 years. Her sister also had hair fall. All nails, finger (Fig. 1) and toe, were rough and ridged. Several were very thin but some were thick. Hair fall was characteristic of a relatively mild alopecia areata.

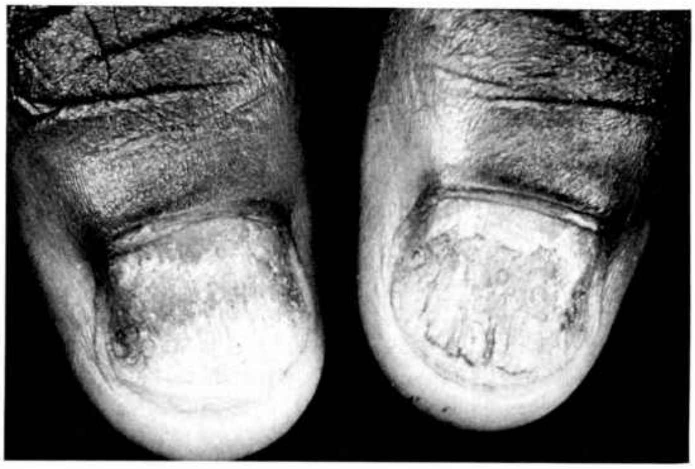
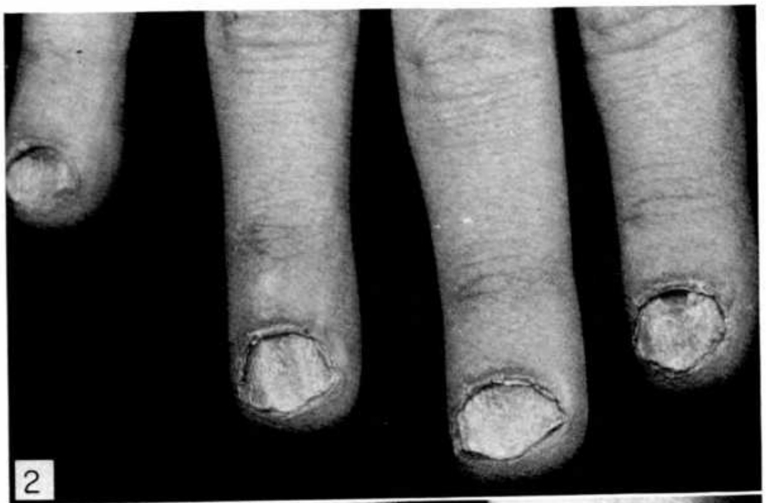


FIGURE 1. Trachyonychia—alopecia areata.

Case 3. M.G. aged 12, male. Changes in finger nails started 2 years previously and toe nails 1 year later. The finger nails were pitted, ridged and some showed koilonychia. Toe nails were more severely affected except for the left fifth toe nail which was spared.

About 6 months after his first attendance he developed diffuse hair fall which was considered to be a diffuse form of alopecia arata. This cleared after a few months and the nails gradually improved so that they were almost clear 3½ years from the date of onset.



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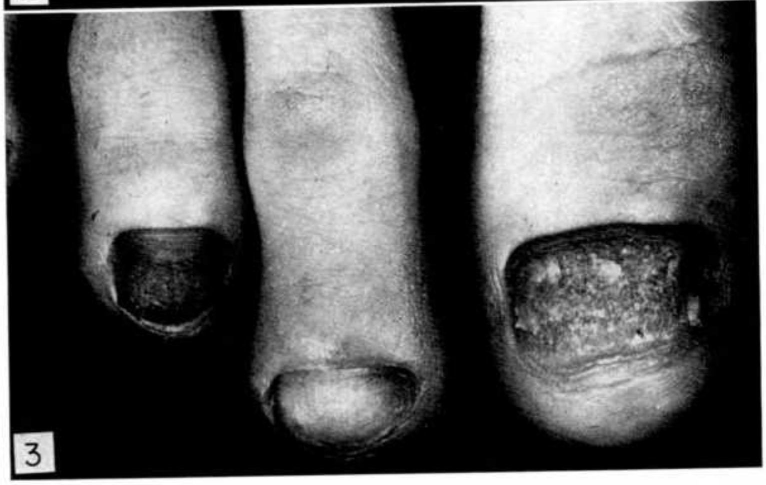

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FIGURES 2 AND 3. Twenty nail dystrophy of childhood.

Twenty nail dystrophy of childhood

Case 4. M.H. aged 8, male. The condition began when he was 3 years of age. Nail splitting was the main complaint. He had no other skin disease. On examination all nails were ridged, although not severely. There was mottling in the half moons.

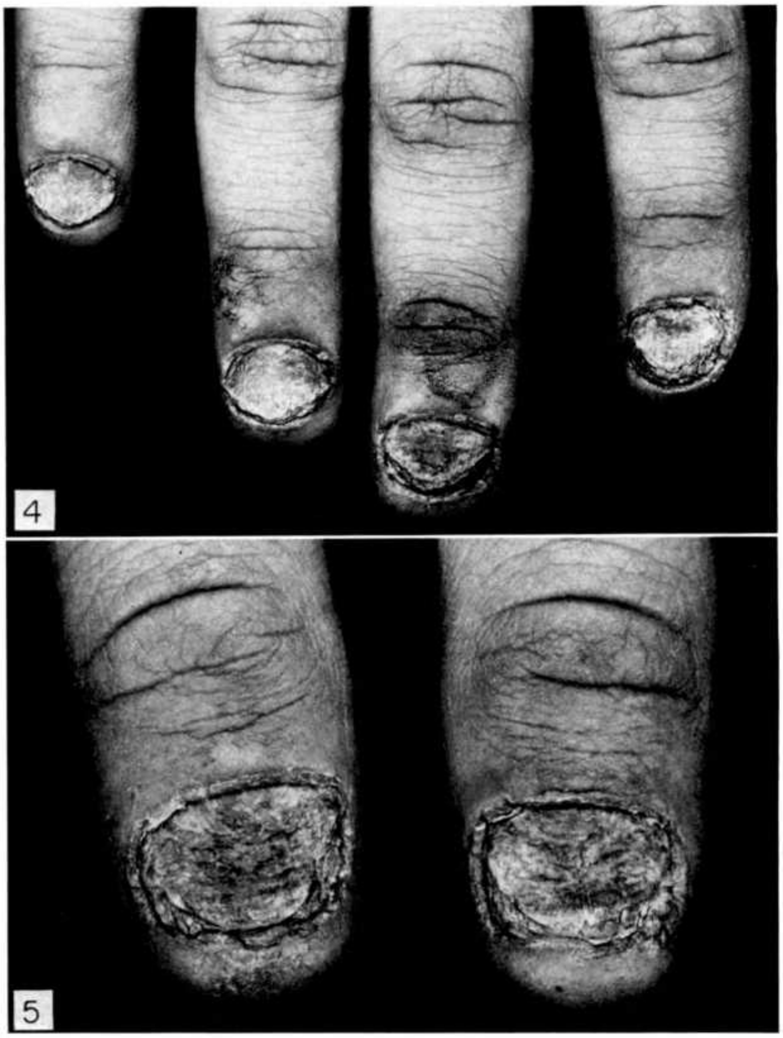
Case 5. A.P. aged 11, male. The condition started 18 months previously. All finger nails were ridged, thick and discoloured and some showed koilonychia (Fig. 2). Both great toe nails and the right third toe nail were thick and discoloured (Fig. 3) but the other toe nails were normal.

The condition began to improve but 6 months later another toe nail became involved.

Severe nail dystrophy

Case 6. S.V. aged 4, male. Eighteen months previously, when he was aged 2, his parents noted his nails becoming thin and curling at the tips. Examination showed all nails very ridged and it appeared that the finger nails were beginning to separate at the base. Six weeks later the finger nails were almost completely lost. The toe nails except those on the great toes were thick. The great toe nails were to a large extent missing. Subsequently, there was partial regrowth of finger nails but the toe nails had not returned after 2½ years.

Case 7. S.S. aged 10, male. The condition started on the finger nails 2 years previously and was just starting on the toes. All nails except the left little toe became dark, pitted and thick (Figs 4 and 5). Some nails showed koilonychia especially the thumb and index finger nails. At one time he developed slight thinning of the hair around the crown but this did not progress. In all other respects he was fit and well. He was observed for 2 years and at that time there was slight improvement in the finger nails.



FIGURES 4 AND 5. Severe nail dystrophy.

Case 8. An adult case: R.B. aged 33, male. The trouble began 1 year earlier after a visit to Mexico. He had lost 2 stones in weight at the time the nails began to change. He also said he had some trouble in the scalp but was otherwise well. On examination there was intense ridging of all finger nails and the tips were broken. The toe nails were less affected but there was mottling of the half moons of the great toes. His scalp appeared normal. He was given treatment by injection of triamcinolone using the Porton gun and there was some improvement. This was repeated on three occasions. He was discharged from observation 3 years after his first attendance, and the nails were by then almost normal. He had received no injections for over a year.

DISCUSSION

This group of patients is difficult to place. The changes in the nails are very real and very distressing to the patients and their parents. Of possible diagnoses, lichen planus seems least likely. Although lichen planus of the nails can occur as a single symptom in childhood it is usually accompanied by pain and when the nail is lost it is with scarring so that the nail cannot regrow. Psoriasis is also a possibility but very unlikely to be the cause. There seems little doubt that the association with alopecia areata is of significance in some cases but Wilkinson *et al.* (1979) did not think so. No explanation of how the association occurs has yet been offered. Some cases have shown only very mild alopecia. Is it possible that the nail changes of alopecia areata can occur in the absence of obvious hair loss? Nail biopsies may be helpful. Wilkinson *et al.* (1979) have recently reported on nail biopsy of one case and thought the changes constitute a distinct histopathological entity. The changes consisted of considerable distortion of the nail matrix with a fairly dense mononuclear inflammatory infiltrate below, and infiltrating into, the matrix epithelium. There was marked spongiosis but no basal cell liquefaction. The nail plate showed parakeratosis, somewhat focal, throughout its length.

The term trachyonychia is a symptom and is no more specific than onycholysis.

ACKNOWLEDGMENTS

I wish to thank the photographic department of St John's Hospital for Diseases of the Skin for the illustrations and my numerous colleagues who continue to refer cases of nail dystrophy.

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Case Reports

Yellow nails and nephrotic syndrome

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SUMMARY

A patient is described who simultaneously developed the yellow nail syndrome and nephrotic syndrome. Treatment of the nephrotic oedema coincided with return of normal nail growth.

Samman & White (1964) first described the yellow nail syndrome. Since then it has become well recognized in association with primary lymphoedema, pleural effusions and other pulmonary manifestations (Emerson, 1966; Beer, Pereira & Snider, 1978). Other associations have also been described, some perhaps fortuitous (Kamatani *et al.*, 1978). We report a case in which yellow nails and nephrotic syndrome caused by glomerulonephritis were associated in the absence of detectable lymphatic abnormalities.

CASE REPORT

A 51-year-old woman presented with an 18-month history of cough, rhinorrhoea, nail abnormalities (cessation of growth, yellowish discolouration and alteration of shape) and ankle swelling. These symptoms started after an upper respiratory infection; cessation of nail growth was noticed about 3 weeks before ankle oedema. There was no past or family history suggestive of lymphoedema.

On examination characteristic changes of the yellow nail syndrome were present—greenish-yellow discolouration, gross thickening, increased curvature, separation from beds and transverse ridging involving all nails (Fig. 1). She also had pitting oedema to midcalf. Investigation revealed heavy proteinuria (6 g/24 h) and a low serum albumin (26 g/l); renal function was otherwise normal and there was no haematuria. Renal biopsy showed a mesangioproliferative glomerulonephritis with immune complex deposition in the glomerular basement membrane (Fig. 2). Bipedal lymphangiography was performed but the lymphatics throughout the lower limbs appeared normal and the subsequent lymphadenogram was also normal.

Within a few weeks of starting treatment with frusemide and spironolactone normal nail growth recommenced and has slowly continued.

COMMENT

While white nails are a well recognized reflection of hypoproteinaemia in nephrotic syndrome

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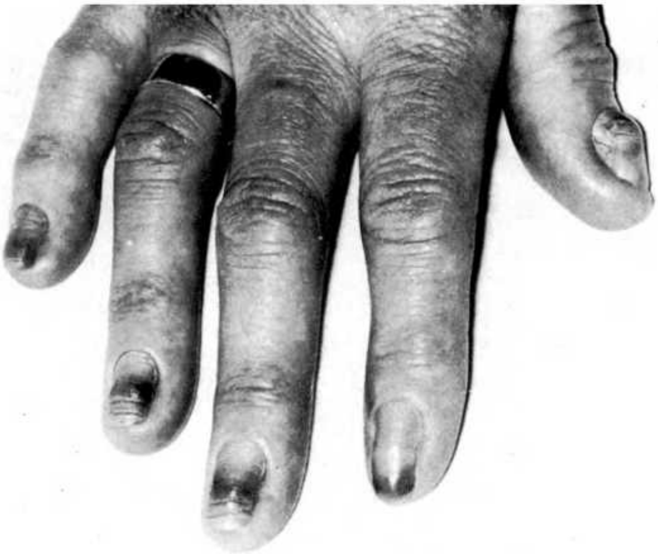

FIGURE 1. Yellow nails on 1 June 1978.

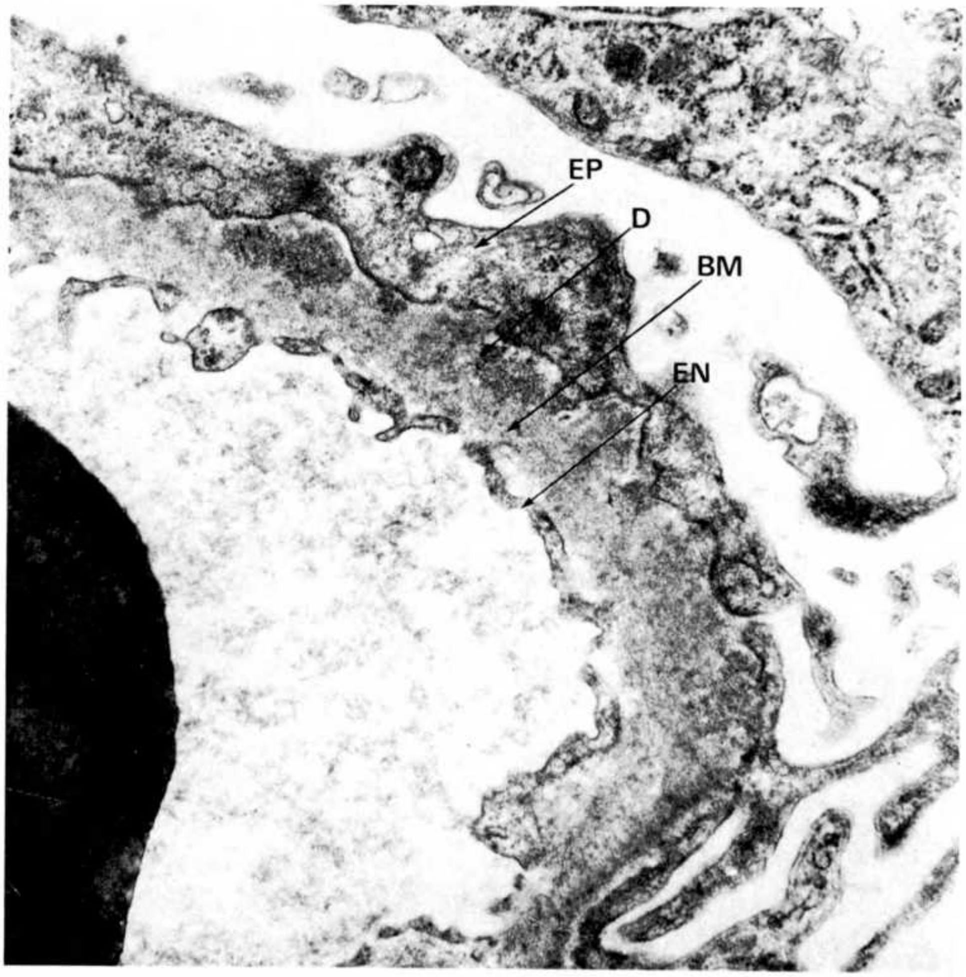

FIGURE 2. Electronmicrograph of a glomerulus showing sub-epithelial immune-complex deposits (D) in basement membrane (BM). EP = epithelium with fused foot processes. EN = endothelium (× 26,000).

(Muehrcke, 1956), yellow nails are generally associated with lymphoedema. Indeed Beer *et al.* (1978) stated that the lymphatic abnormality is paramount and the discoloured nails are merely a superficial indicator of an underlying defect. Lymphangiography did not show any lymphatic abnormality in our patient, although this does not exclude a minor degree of lymphatic hypoplasia which only interfered with nail growth when nephrotic oedema was superimposed. Although there are reports of hypoproteinaemia and the yellow nail syndrome, nephrotic syndrome without detectable lymphoedema has not been reported. Radenbach & Wundschock (1974) describe a case in which nephrotic oedema due to glomerulonephritis occurred with the yellow nail syndrome but this patient appeared to have multisystem disease and widespread lymphangiopathy.

A further point of interest in our patient is the return of normal nail growth. It is generally thought that any improvement in the nails is spontaneous but in this case diuretic treatment more than a year after the onset of yellow nails and oedema resulted in resumption of normal nail growth.

ACKNOWLEDGMENT

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Complement abnormalities in diffuse plane xanthomatosis with paraproteinaemia

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SUMMARY

Paraproteinaemia may be associated with xanthomatous skin deposits and these can arise in the absence of elevated lipid levels. Two cases of benign monoclonal gammopathy with diffuse plane xanthomatosis are reported. Case 1 exhibited hypolipidaemia and a functional deficiency of C1 esterase inhibitor. Case 2 showed a normal lipoprotein profile, abnormal platelet aggregation, and a cutaneous vasculitis with evidence of complement consumption via the classical pathway. The significance of these abnormalities is discussed.

The term 'diffuse plane xanthomatosis' indicates the presence of widespread xanthomatous skin deposits in which the lesions are typically flat or slightly elevated and vary greatly in size, often producing large areas of orange or yellow discolouration. The eruption is distinctive, and is frequently associated with disorders of the lymphoproliferative series, particularly myeloma and other 'M' component diseases (Lynch & Winkelmann, 1966). This group is of particular interest, since xanthoma formation seems to occur independently of serum lipid levels, the majority of cases being normolipaemic. Other types of xanthomatosis have been described in association with paraproteinaemia, including cases of tuberous xanthomata (Wilson *et al.*, 1975) eruptive xanthomata (Roberts Thompson *et al.*, 1975) and xanthoma disseminatum (Maize, Ahmed & Provost, 1974). It is important to distinguish these groups as the mechanisms involved are liable to be different. The patients described below illustrate the association of diffuse plane xanthomatosis and paraproteinaemia.

METHODS

Complement studies were carried out by the Department of Immunology at East Birmingham Hospital. The CH50 titre was estimated by the method of Mayer (Mayer, 1961), and functional C4, using the lysis of sensitized sheep red cells by C4 deficient guinea-pig serum. Functional C2 was

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estimated by the method of Thompson (Thompson, 1979), and immunochemical analysis of C1q, C4 and C2 was carried out by the single radial diffusion test using specific antisera.

Paraprotein studies were carried out at the Westminster Protein Reference Laboratory. Samples were electrophoresed on cellulose acetate (Shandon Instruments, Camberley, Surrey) using a multi-applicator (Shandon Instruments), then stained and dried (Kohn, 1973). Electrophoretic separations were scanned in a Zeiss scanning densitometer and the paraprotein concentration calculated as a percentage of the total protein estimated by a modified biuret technique (Doumas, 1975). Precipitating antibodies with antiheparin or antilipoprotein activity were looked for using Ouchterlony double diffusion and laser nephelometry.

Lipid studies were carried out by the Westminster School of Medicine, Biochemical Research Laboratory. Lipoprotein electrophoresis was carried out on cellulose acetate and the separations examined in a Gelscan automatic recording and integrating scanner.

CASE REPORTS

Case 1

Mrs E.I., a 66-year-old Caucasian female, presented in 1975 with a 2-year history of orange skin deposits. These had appeared originally around the eyelids. Further lesions had developed on the chest, upper lip and within the mouth. Her past medical history included pulmonary sarcoidosis which had been treated in 1950 with streptomycin for 2 years. For the past 8 years she had suffered intermittently with angina. Her therapy on admission was digoxin and trasicor.

Investigations. Haemoglobin—14·3 g/l; WBC—5,100/mm^3; ESR—27 mm/h; total protein—65 g/l, serum albumin—30 g/l. Immunoglobulins: IgG—13·7 g/l; IgM—0·71 g/l; IgA—1·09 g/l. Serum protein immunoelectrophoresis revealed an IgG Kappa paraprotein at a concentration of 10 g/l. There was no Bence Jones proteinuria. A skeletal survey was normal. A bone marrow revealed an increased plasma cell population with some nuclear atypicality, but no definite features of myeloma. Serum lipoprotein electrophoresis showed no evidence of hyperlipoproteinaemia. However, an abnormal beta lipoprotein sinking to a very low alpha lipoprotein was present at a level of 52 EFR Index Units. Prebeta, beta and alpha lipoproteins were present at concentrations within the normal range (198, 71 and 26 EFR Index Units respectively). Serum cholesterol was depressed (4·81 mmol/l), serum triglycerides 1·36 mmol/l, phospholipids 3·01 mmol/l. Complement studies revealed an elevated C3 (2·13 g/l), a normal C4 (0·46 g/l), and a normal C1 esterase inhibitor level by immunochemical estimation (0·38 g/l). The functional C1 esterase inhibitor activity was only 50% of normal. Ouchterlony double diffusion and laser nephelometry did not reveal the presence of precipitating antibodies with antiheparin or antilipoprotein activity. Skin biopsy showed a diffuse xanthomatous infiltrate throughout the dermis. Direct immunofluorescence was negative for all conjugates, including IgG and complement. Stains for amyloid were negative.

The following investigations were normal or negative: urea and electrolytes, liver function tests, thyroid function tests, ANF and autoantibodies, rheumatoid factor, cryoglobulins, chest X-ray.

Case 2

Mrs S.C., a 51-year-old Caucasian female, presented in 1977 with yellow skin deposits. She is a known case of mixed mitral valve disease, having had rheumatic fever at the age of 13. In 1974 she developed effort dyspnoea in association with the onset of atrial fibrillation. Antihypertensive therapy was instituted in 1975. Drug therapy at the time of referral was digoxin, moduretic, methyldopa and warfarin.

Examination revealed extensive, yellow skin deposits situated on the thighs, within the antecubital fossae and around the axillae. Petechiae were visible within these lesions, but the patient was not sure for how long they had been present. She was in atrial fibrillation, had a blood pressure of 220/120 mmHg and a mild degree of mitral stenosis.

Investigations. Haemoglobin—15·9 g/l; WBC—6,800/mm^3; platelets—221,000/mm^3; ESR—84 mm/h with rouleaux formation on the film. Serum protein—88 g/l; albumin—38 g/l. Serum IgG—35·0 g/l; IgA—1·8 g/l; IgM—0·7 g/l. Serum immunoelectrophoresis revealed a paraprotein IgG lambda present at a concentration of 24·4 g/l, subclass IgG1. Immunoelectrophoresis of concentrated urine revealed free lambda chains. Skeletal survey was normal. A sternal marrow revealed 8% mature plasma cells with some cytoplasmic vacuolization, but no definite features of myeloma. Serum lipoprotein electrophoresis was normal, cholesterol—6·2 mmol/l, triglyceride—1·5 mmol/l. Ouchterlony double diffusion and laser nephelometry failed to detect the presence of precipitating antibodies with either antiheparin or antilipoprotein activity.

Complement studies: total haemolytic complement (CH50)—20·5 u/ml (normal 25–45 u/ml). C1q functional—55% standard (normal 70–125%). C1 esterase inhibitor-immunochemical 0·18 g/l (normal 0·18–0·26 g/l). C1 esterase inhibitor functional 90% standard. C4 immunochemical 0·02 g/l (normal 0·15–0·54 g/l). C4 functional 10% standard. C2 functional 53% standard (normal >80). C3 immunochemical 1·88 g/l (within normal limits). Complement studies were carried out on the patient's father and her two daughters. C3 and C4 levels were normal.

Platelet studies: platelet count 172 × 10^9/l; platelet adhesiveness moderately decreased; ristocetin platelet aggregation normal; secondary platelet aggregation with ADP was inhibited. Total immunoglobulin coating platelets was 537 ng/10^6 cells (normal >18 ng/10^6 cells) of which 122 ng was IgG.

Clotting studies: BCR 1·8 :1 (patient on warfarin); KCCT 46·5 (control 41), bleeding time normal at 3 min. Factor VIII assay normal at 180%; clot retraction normal. Platelet agglutinins, leukocyte agglutinins and incomplete platelet antibodies were all negative.

Autoantibody studies: antinuclear factor +, smooth muscle antibodies ++, thyroid microsome haemagglutination titre 1: 1600.

Skin biopsy: a perivascular infiltrate was present around upper dermal vessels. This consisted of lymphocytes with a few polymorphs and some extravasated red cells. Xanthomatous deposits were present throughout the dermis but particularly located around the upper dermal vasculature. Direct immunofluorescence was negative for all conjugates including IgG and C3.

The following investigations were normal or negative: urea and electrolytes, liver function tests, thyroid function tests and cryoglobulins.

DISCUSSION

Investigation of patients with paraproteinaemia and xanthomatosis is of particular interest since analysis of the properties of the paraprotein may clarify the mechanism of lipid deposition. Two properties which could readily interfere with lipid metabolism have been identified, namely anti-heparin and anti-lipoprotein activity. Paraproteins with anti-heparin activity may interfere with lipid metabolism since heparin is important for the activity of certain enzymes involved in lipoprotein catabolism particularly lipoprotein lipase (Glueck, Mackenzie & Glueck, 1972). Such cases exhibit hyperlipidaemia, atherosclerosis and nodular xanthomatosis if skin involvement is present (Wilson *et al.*, 1975). Diffuse plane xanthomatosis has not been described in association with anti-heparin activity. Neither of our patients had features of this syndrome, nor was anti-heparin activity evident when tested *in vitro*.

Paraproteins with lipoprotein binding activity have been extensively investigated by Beaumont, Jacotot & Beaumont (1965), Beaumont, Jacotot & Vilain (1965a, b), Beaumont (1965, 1966, 1967, 1968, 1969, 1970), Jacotot, Nguyer-Trong & Beaumont (1965) and Beaumont, Beaumont & Antonnulie (1970) and the association of this property with cutaneous xanthomatosis has been recently reviewed by Taylor *et al.* (1978). Twenty-one examples are cited in which complexing of serum lipoproteins and paraproteins has been demonstrated in patients with myeloma and xanthomatosis. Diffuse plane xanthomatosis occurred in fourteen of the fifteen cases in which the xanthoma type is recorded, the one exception displaying tuberous xanthomata with markedly elevated serum lipids. Lipoprotein–paraprotein complexes were separated by ultracentrifugation in fourteen of the fifteen cases in which the procedure is documented. There is, therefore, good correlation between the presence of lipoprotein-paraprotein complexes and diffuse plane xanthomatosis.

It is not certain how the formation of such complexes leads to xanthoma deposition in the dermis. It is possible, however, that deposition occurs in a manner analogous to immune-complex vasculitis. The rate of lipid deposition would then be determined amongst other things by the properties of the complex rather than the serum lipid levels and this would account for the observation that most cases of diffuse plane xanthomatosis are normolipaemic. In this context it is significant that some of the lipoprotein-paraprotein complexes have been cryoprecipitable (Beaumont, 1969; Kodama, Nakagawa & Tanioku, 1972; Wilson *et al.*, 1975; Taylor *et al.*, 1978).

These observations are supported in our two patients, both of whom had typical diffuse plane xanthomatosis with no other varieties of xanthomata. Case 2 had a normal lipoprotein profile, whilst Case 1 had hypolipidaemia with an abnormal sinking lipoprotein. In neither of our patients were we able to demonstrate lipoprotein binding *in vitro*, but the methods used were relatively crude and there was evidence suggestive of immune-complex formation *in vivo*.

Thus, Case 2 showed complement abnormalities consistent with complement consumption via the classical pathway. Levels of C1q, C4 and C2 were all depressed, C4 being particularly low (10% of normal). This is unlikely to be an inherited C4 deficiency since no relative had C4 deficiency and the paraprotein belonged to a subclass known to activate complement via the classical pathway (IgG1). A similar complement profile has been reported in association with an IgM lipoprotein cryoglobulin in which the ability of the IgM to activate C1 at 37°C was dependent upon the presence of lipoprotein and the formation of IgM lipoprotein complexes (Linscott & Kane, 1975).

The presence of purpura in this patient is also of considerable interest. In the absence of amyloid, purpura in association with paraproteinaemia may be due either to platelet abnormalities or to a vasculitis due in turn to deposition of immune complexes or paraprotein aggregates. Platelet studies in our patient demonstrated that secondary platelet aggregation with ADP was inhibited, and that surface coating of the platelets with immunoglobulin was greatly increased. However, there was no evidence of a generalized bleeding diathesis, and the petechiae were confined to those areas of skin showing xanthomatous infiltration. Furthermore, the histology showed an upper dermal perivascular infiltrate containing polymorphs as well as extravasated red cells. It is therefore likely that the purpura seen in this patient represents a complement mediated vasculitis, and the distribution of the vasculitis corresponds to that of the xanthomata because they are both mediated by deposition of lipoprotein-paraprotein complexes.

In Case 1 the hypolipidaemia provides circumstantial evidence of complex formation, since rapid clearing of such complexes has been proposed as the mechanism underlying the low levels of serum lipid seen with myeloma (Riesen, Noseda & Butler, 1971). The finding of a low functional C1̄ esterase inhibitor activity in this case is not readily explained and does not provide evidence for the presence of immune complexes. However, it is unlikely to be a chance finding since Jordon has reported a similar case of diffuse normolipaemic xanthomatosis and paraproteinaemia with markedly reduced C1 esterase inhibitor activity, and low levels of C1q, C4 and C2 (Jordon *et al.*, 1974).

Many of the cases of diffuse plane xanthomatosis reported by Beaumont exhibited hyperlipidaemia as well as complexing. Reduced clearance of these complexes was therefore suggested as the mechanism underlying the hyperlipidaemia as well as the xanthomata. There are, however, a number of objections to this hypothesis. First, Riesen *et al.* (1971) have demonstrated that such complexes are usually cleared more rapidly than lipoprotein alone. Secondly, in cases where diffuse plane xanthomatosis is associated with hyperlipidaemia, the lipid composition of the xanthoma does not correspond with the serum lipids (Kodama *et al.*, 1972). Thirdly, it has been shown that the complexing and the hyperlipidaemia involve different lipoprotein fractions (Marien & Smeenk, 1975). Finally, therapy for the myeloma often corrects the hyperlipidaemia but has no effect upon the xanthomatous lesions (Moschella, 1970).

In conclusion, lipoprotein binding is the only property that has been consistently demonstrated in patients with paraproteinaemia and diffuse plane xanthomatosis. Most cases are normolipaemic and other types of xanthomata are only seen with a paraproteinaemia if hyperlipidaemia is also present.

These cases illustrate the range of immunological and haematological abnormalities that may occur with paraproteinaemia and indicate that immune complex formation underlies the lipid deposition seen in diffuse plane xanthomatosis.

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Comedo naevus of the palm—a sweat duct naevus?

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SUMMARY

A 9-year-old boy is described with keratotic pits on the left palm, present since birth, which clinically resembled those seen in comedo naevus. Histology and scanning electron microscopy of skin surface replicas showed the lesions to be large, abnormal sweat ducts. It is suggested that comedo naevi are not necessarily pilosebaceous in origin and that in our case the term sweat duct naevus may be more appropriate.

Comedo naevus is an uncommon developmental abnormality which consists of clusters of tiny pits each containing a horny keratin plug. Lesions are usually present at birth, occur most commonly on the face and are unilateral with a linear or zosteriform distribution. The majority of patients have no other associated defect (Rook, 1972).

Comedo naevi are generally presumed to be pilosebaceous in origin (Beerman & Homan, 1959; Nabai & Mehregan, 1973) and if this is true then it is hardly surprising that lesions on the palms or soles, areas normally devoid of hair, are very rare. A few patients with palmo-plantar involvement have been reported, however, some with typical lesions on other parts of the body (Klein, 1917; Wood & Thew, 1968; Leppard & Marks, 1973; Leppard, 1977).

A patient is described with characteristic pitted lesions on the palm of the left hand. The histology and scanning electron microscopy of skin surface replicas both suggested that the lesions were derived from abnormal sweat ducts.

CASE REPORT

Clinical and microscopic findings

The patient was shown to the Dermatology Section of the Royal Society of Medicine in November 1977.

A.E., a male aged 9, was born with a linear group of pits on the palm and middle finger of the left hand (Fig. 1). The lesions have changed little since birth and the skin elsewhere is unaffected. His parents and sister are normal. Each of the pits contains a plug of keratin up to 2 mm in length and with the help of a hand lens it can be seen that they are arranged along the skin surface ridges within the lines of sweat pores.

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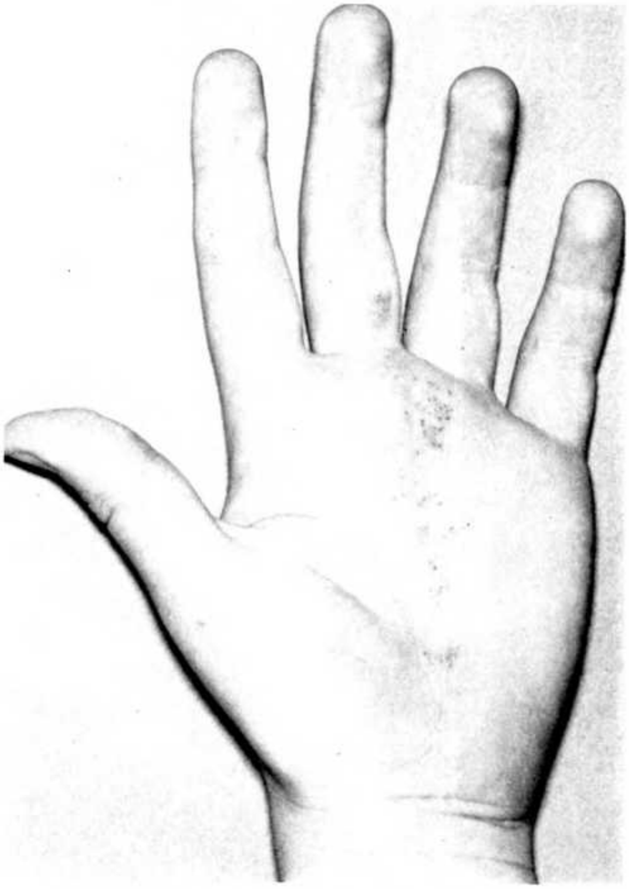

FIGURE 1. Comedo-like lesions on the left palm.

Silicone rubber/Araldite skin surface replicas were made of the affected palm using the method of Johnson, Dawber & Shuster (1970), coated with gold and examined in a Cambridge S2 Stereoscan. Scanning electron microscopy confirmed that the pits were situated on the dermatoglyphic ridges within the lines of normal looking sweat pores. The keratinous plug in some pits appeared dome-shaped (Fig. 2), while in others it protruded in a worm or hair-like manner (Fig. 3). Most of the pits had a large sweat bubble exuding from them which appeared on the Araldite replicas as a smooth spherical structure.

Histology

The pits consisted of dilated abnormal epidermal sweat ducts, many containing a parakeratotic filiform plug. Some of the plugs possessed a slit-like cavity, possibly representing a sweat duct lumen. In others, however, a sweat duct lumen was present at the side (Figs 4 and 5).

At the base of the plug the granular layer was absent and there were epidermal cells with vacuolated cytoplasm. Deep to this, the upper part of the dermal sweat duct was both dilated and hyperplastic. The eccrine glands were normal apart from some dilatation.

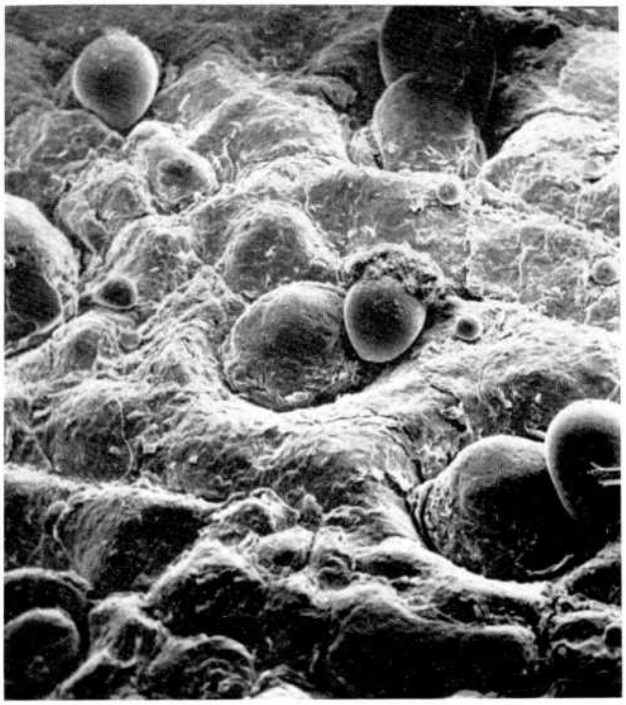

FIGURE 2. Scanning E.M. of Araldite skin surface replica. The dermatoglyphic ridges in this view are distorted by pits which contain relatively flat keratotic plugs. Large sweat bubbles (the smooth spherical structures) can be seen exuding from the pits (× 55).

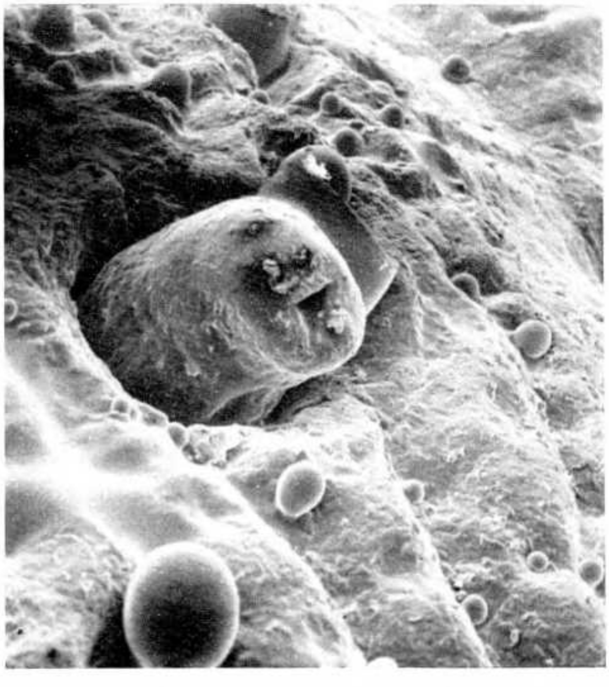

FIGURE 3. A pit containing a hair-like plug of keratin and a large sweat bubble (which has fractured during processing) (× 66).

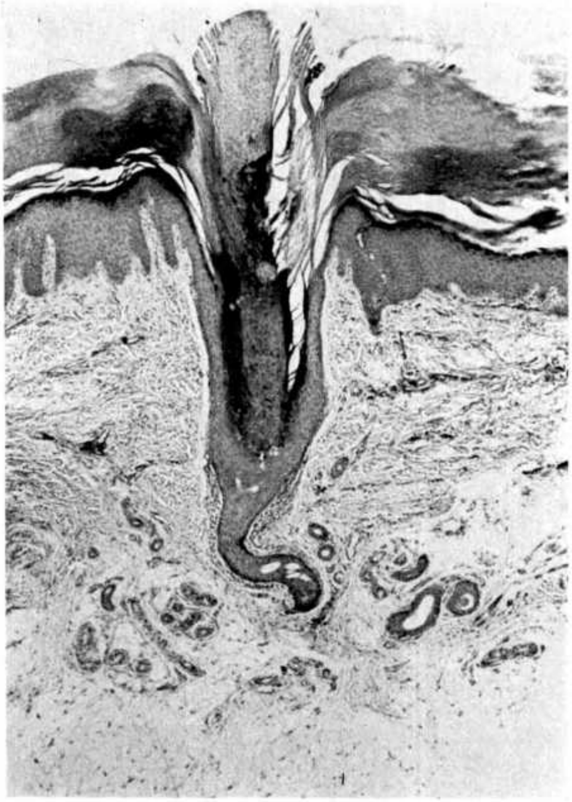

FIGURE 4. Biopsy of a pit showing a dilated sweat pore with a filiform parakeratotic plug. The duct is hyperplastic and contains a lumen which, in this section, extends as far as the base of the plug (H & E, × 50).

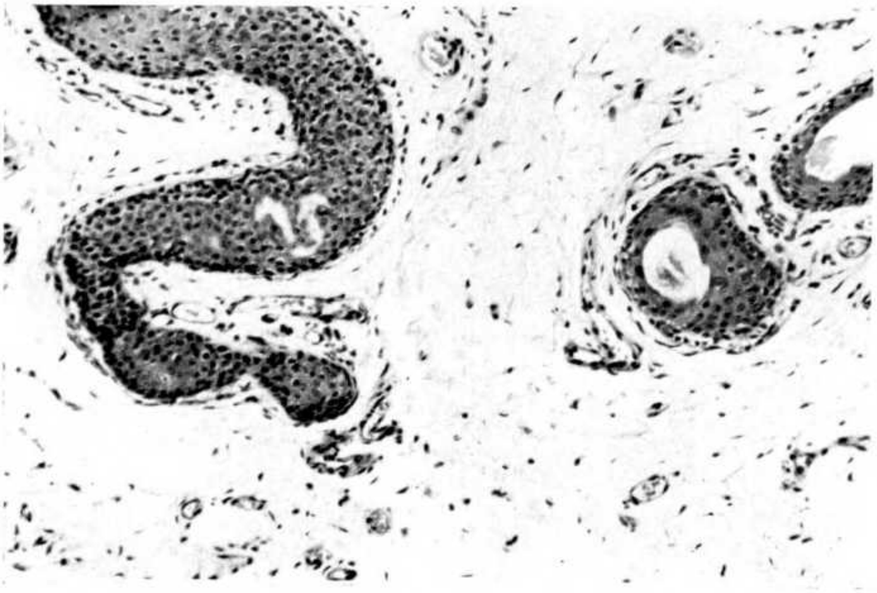

FIGURE 5. The ducts terminated in the dermis at sweat gland level. Secretion can be seen within the lumen (H & E, × 200).

It was not possible to demonstrate direct continuity between eccrine coil and the sweat ducts, but in several the duct lumen contained secretion, presumably sweat. Some of the lesions in which a lumen could not be seen seemed to consist of solid cords of cells. The rest of the epidermis was mildly acanthotic, but there were no areas of parakeratosis other than in the sweat ducts. There was no significant inflammatory infiltrate.

DISCUSSION

Comedo naevi are thought to be hamartomatous malformations of the pilosebaceous apparatus (Beerman & Homan, 1959; Nabai & Mehregan, 1973). Biopsies of lesions on hair-bearing skin show dilated, sometimes almost cystic, follicular-like canals containing keratotic plugs. The epithelial wall is thin but may also show irregular thickenings which project into the surrounding dermis. Rudimentary hairs and sebaceous glands may be found in early lesions but are usually absent. Leppard (1977) reported a patient with an extensive comedo naevus who developed multiple trichilemmal cysts.

Reports of four patients with comedo naevi on the palm or sole have been found but in only one of them is the histology described (Wood & Thew, 1968). A thick follicular-like structure was present which narrowed in the mid-dermis and terminated deeper, at the level normally occupied by sweat glands. The structure contained a central lumen filled with keratin; no hair shafts or sebaceous glands could be seen. The authors, who were unable to find sweat glands in the sections examined, concluded that the lesion was a poorly differentiated hair follicle. It is of interest that some sections from our patient's lesions showed neither duct lumen nor sweat glands and therefore resembled those described by Wood & Thew (1968).

In contrast the histological and scanning electron microscopy findings in our patient suggested that the lesions were giant sweat ducts which superficially showed abnormal keratinization. Scanning electron microscopy of skin surface replicas confirmed the clinical impression that the pits were on the surface ridges where sweat pores are normally found. Although we were unable to demonstrate any connection between the duct-like lesions and adjacent sweat glands, sweat bubbles (much larger than normally seen) could be seen exuding from the pits indicating that the giant ducts were functioning and presumably in continuity with sweat glands. A convoluted lumen, apparently containing secretion, was seen on histology which with the help of serial sections could be traced throughout the lesions extending from deeper dermis to the tip of the filiform plug.

Pitted lesions on the hands, similar to those reported here, may be found in punctate and linear porokeratosis of Mibelli. Like comedo naevus, lesions may be present at birth or appear in childhood (Rahbari, Cordero & Mehregan, 1974, 1977). The parakeratotic cornoid lamella that is characteristic of porokeratosis usually arises over sweat ducts but sometimes occurs in the epidermis between adnexal structures. Our patient's lesions differed both clinically and histologically from those described by Rahbari *et al.* (1974, 1977). They did not have a raised keratinous rim and parakeratosis was found only within sweat ducts. The mild inflammatory dermal infiltrate usually seen in porokeratosis was absent. The distinctive giant sweat ducts, found in our case, are not present in porokeratosis of Mibelli.

The evidence presented here suggests that our patient's lesions are derived from abnormal sweat ducts and that the term sweat duct naevus may be more appropriate. The word comedo, although originally alluding to a worm or maggot-like appearance, now implies pilosebaceous involvement. Perhaps scanning electron microscopy techniques and simple tests of eccrine function, such as the starch iodide reaction, might be useful in determining the origin of comedo naevi when conventional histology is unhelpful.

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Brief Communication

A simple aid for the application of skin preparations

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With improved methods for treating skin disease it becomes increasingly important that the therapy prescribed is actually used by the patient. Treatment failure may occur for as simple a reason as the area of the body to be treated either cannot be reached or requires a difficult manoeuvre before the skin preparation may be applied. This is true particularly of the human back which continues to provide a challenge to topical therapy.

The young, otherwise healthy, patient might regard treating a dermatosis over the back as awkward but manageable, but for the less supple or the infirm the attempt at therapy may rapidly become more disheartening than the disease itself.

To try to overcome this a simple plastic skin applicator has been designed (Fig. 1). It is light (wt. 115 g) and has a roller head, 3 cm wide, which is easily detached and so may be washed. Most areas of the body including the back can be reached with a reasonable degree of accuracy.

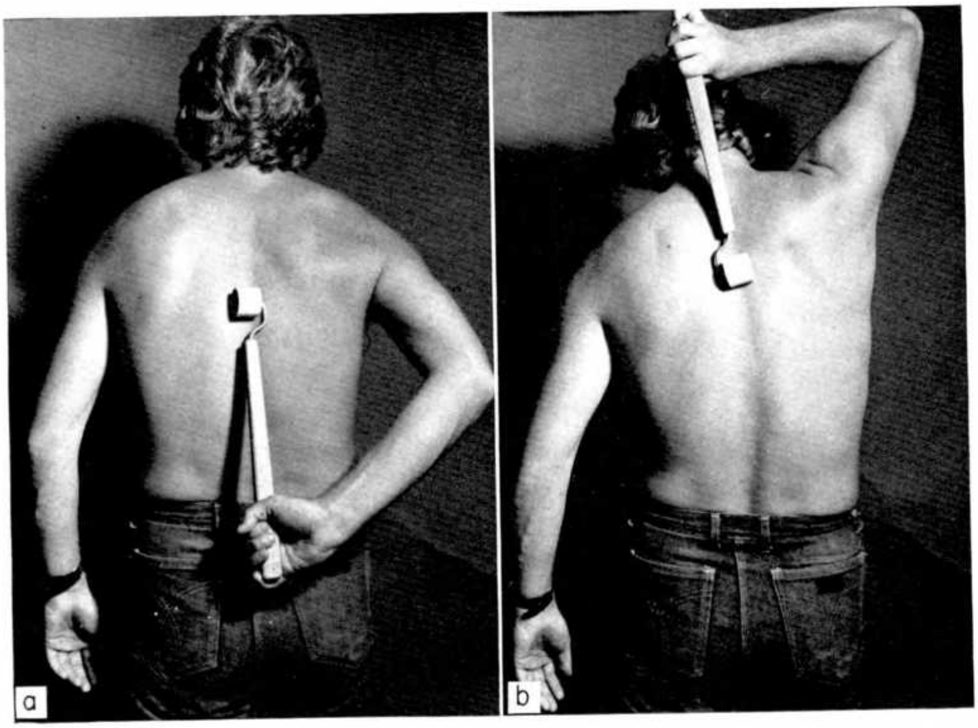

FIGURE 1a AND b. Demonstration of the use of the applicator on the back of a patient.

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A preliminary trial has confirmed that creams, ointments and lotions are applied with ease but pastes tend to adhere to the roller surface as indeed they do to wooden spatulae, which are the present means of application. Patients find the applicator aesthetically acceptable and quote the added advantage that it provides independence with therapy which previously they had, only reluctantly, relinquished.

ACKNOWLEDGMENT

I am grateful to Mr L.R. Richmond for his help in the design of the handle.

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Comment

PSORIASIS: A DYNAMIC DISEASE

At the moment it is generally accepted that the regulation of cell division and differentiation is significantly altered in psoriatic keratinocytes. Observations at the ultrastructural level (Orfanos *et al.*, 1976) have suggested that the fault which is responsible for these abnormalities may be located in the plasma membrane, in particular involving the glycocalyx. We have tried to quantify these abnormalities by studying the isolated keratinocytes of psoriatics and normal subjects. As it seems likely that in psoriatics the glycocalyx is indeed different from normal, we have also tried to correlate the changes at the molecular level with the clinical aspects by using an intermediate method (flow cytophotometry), by which abnormalities in the cell cycle can be studied at the cellular level.

We are well aware of the limitations of our research. In the first place we considered only the epidermis. We believed that here would be found the key for psoriasis. We were further unable to separate in our experiments the proliferating from the differentiating cells; we could only estimate their relative contributions in separate preparations.

In the investigations on the plasma membrane it is of the utmost importance to avoid extensive changes in the membrane resulting from the methods used for preparing the isolated cells. In the course of the experiments simple trypsinization was modified by the use of dithioerythritol to cleave the desmosomes and the addition of calf serum as trypsin inhibitor before mechanical disruption. The cells remain viable as appears from their ability to exclude Trypan blue and to grow in tissue culture.

Scanning electron microscopy shows progressive changes in the morphology of the glycocalyx accompanying the differentiation process. The cells increase in size while the microvilli of the glycocalyx shrink as the keratinocytes approach the skin surface. Keratinocytes from psoriatic lesions are morphologically distinguishable from those of the epidermis of normal controls. They showed at all levels microvilli which were at least three times longer than normal. Similar but less pronounced changes were usually observed in cells prepared from the clinically uninvolved epidermis of psoriatics.

The sugar composition of the external surface of the glycocalyx was studied by Gommans *et al.* (1979a). He calculated the number of binding sites for various lectins, which are very specific markers for the presence of sugars. He found for normal skin keratinocytes $6{\cdot}5 \times 10^7$ binding sites per cell for concanavalin A (indicating glucose and mannose) and $21{\cdot}5 \times 10^7$ binding sites per cell for wheat germ agglutinin (indicating N-acetyl glucosamine). In psoriatics the levels increased to $19{\cdot}5$ and $39{\cdot}8 \times 10^7$ respectively. Clinically uninvolved epidermis yielded intermediate values. In contrast, the affinity of the lectins for the cell surface appeared to be diminished, while the ratio between the binding capacity for both lectins in psoriatics was also different from normal. Both facts indicate that the difference in sugar content of the glycocalyx is not merely a non-specific change due to the change in size but is of a more fundamental nature.

The synthesis of glycoconjugates in the plasma membrane was studied by measuring the uptake of radioactive sugars into suspensions of isolated keratinocytes. The rate of uptake of four sugars (L-fucose, N-acetyl glucosamine, D-galactose and D-mannose) was in the order: normal < uninvolved psoriasis < psoriatic lesions. After 22 h pre-incubation the sugar uptake of normal cells remained at the same level, but fell dramatically in the psoriatic keratinocytes. The glycosyl-transferase activity (indicated by the percentage incorporation into glyco-conjugates) appeared to be similar for normal and psoriatic keratinocytes except in the case of L-fucose, which was at about twice the normal

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level in psoriasis. This observation supports the concept that the carbohydrate composition of the glycocalyx is abnormal in psoriasis.

Cyclic AMP levels were determined in the isolated keratinocytes by Gommans *et al.* (1979b). They found maximal cAMP levels in the basal layers falling to lower levels in the upper layers. It was found that keratinocytes prepared from psoriatic lesions show a reduced resting level of cyclic AMP and also a diminished response to adrenaline stimulation. Taking these observations together we see a number of changes in the plasma membrane of the psoriasis patient, both in the lesions and in the normal appearing skin. During the investigations it appeared that the differences within one lesion and between various lesions in one patient are smaller than those from patient to patient.

We see also a very gradual transition in the qualities of the plasma membranes of normal skin to the lesional skin. We might therefore provisionally conclude that psoriasis is a disease of the whole skin, and that everybody has his own psoriasis, determined by the intensity of the genetically induced abnormalities of the plasma membrane. We propose that our findings all point in the direction of a destabilization of the epidermis.

The enlarged villi of the glycocalyx imply a diminution of contact inhibition. The low content of cAMP in the cell enhances the tendency to proliferate. The changes in the sugar composition in the glycocalyx will influence the behaviour of hormone receptors, immunoreceptors and membrane transport. The loss of stability makes the epithelium more prone to influences from the outside world and also from the inside.

A first effect of these influences as a whole will be on the level of the steady state in which the epidermis normally operates. The steady state, notwithstanding the continuous activity of the basal cells, is attained by a cybernetic system which operates at various levels.

At the level of the basal cells we have the negative feedback of chalones or growth inhibitors, which are synthesized by the proliferating cells. In the S phase the basal cell synthesizes DNA and little chalone, while its rate of synthesis diminishes further as the cell enters the G2 phase. In this phase the main metabolic investment by the cell is for spindle construction. The amount of chalone falls still further, passes the critical level and mitosis occurs. The cell now enters G1 and begins to build up cell structures and the synthesis of chalone begins anew. The main complication for this very simplified model is that the basal cells are all in different stages of the cell cycle and the effect of the chalone is different over a large number of cells.

Under normal conditions this means that statistically the same ratios of cells will always be found in the various phases of the cycle. This comes about because the cycles all run asynchronously and the total concentration of the chalones is relatively constant. If by changes at the level of the glycocalyx (determined through the Golgi complex by the DNA in the nucleus) a diminution occurs of the chalone content, the proliferative activity of the basal cells will be heightened. The thickness of the epidermis will then increase till so much chalone is produced that a new steady state, be it at higher level, is attained. Such a situation could develop if the G1 phase in which most chalone is produced should become shorter. The change in regard to the normal situation would be only that the number of cells per square millimetre, i.e. the thickness of the skin, would increase, while no alterations of the stratum corneum would occur. In histological terms we would see acanthosis and hyperkeratosis. This is in fact what we see in the very mild form of psoriasis in patients with small lesions on the elbows, outer ear or head. What we might call psoriasis mitis.

It is possible to investigate the phase partition of epithelial cells by means of flow cytophotometry (Bauer & Mali, 1975). A sample of isolated keratinocytes (200–800,000 for a field of 7 mm^2) after staining the DNA with a fluorochrome, is automatically counted for the DNA content of the individual cells. As we cannot at the moment distinguish between proliferating and differentiating cells, we can only make an estimate of their relative numbers. Bauer & Mali (1975) found in hundreds of estimations in normal skin for the various phases: for G1 97·4%; for S 1·8% and for G2+M 0·9%. In an average psoriasis plaque one will find for G1 88·5%; for S 8.5% and for G2+M 3%. In this case however the total number of cells per mm^2 will be four times as high as in the normal control. The normal appearing skin of the psoriatic patient shows values close to normal but significantly different.

In the more acute forms of psoriasis—such as the circinate and annular erythemous forms, unstable psoriasis and psoriasis pustulosa—the precentage of cells in the S phase will rise to 40–60%, be it that the relation of the proliferating-differentiating pool will also be severely disturbed. The most marked aberrations are seen in impetigo herpetiformis where up to 50% of all cells are observed in the S phase at the culmination of the process, returning to nearly normal levels when the desquamative process has passed. Similar sudden changes were also seen in psoriasis lesions which were treated with dithranol, Röntgen rays or PUVA. In all cases a high percentage of cells accumulated in the S phase after dithranol, in the G2 phase after Röntgen rays and PUVA, and disappeared abruptly when the lesion healed. Reviewing our observations from flow cytophotometry we can thus confirm our first impressions: everybody has his own psoriasis involving the whole skin and there is a step by step transition from normal to very pathological.

How do we now explain the clinical lesions with the biochemical and flow cytometric observations? Just because of the gradual transition we are convinced that it is necessary to distinguish meticulously between the various forms of psoriasis (Fig. 1 and Table 1).

If we assume the normal skin has 10,000 epithelial cells per layer over a surface of 1 mm^2 and to be four layers thick, in total 40,000 cells, the absolute number of cells in the S phase at the level of the stratum basale (one layer thick) will be about 700, the cells in G2 and mitosis about 350. In the normal-appearing skin of psoriasis perhaps two layers of cells are proliferating and two differentiating. The total number of cells might be 1·5 times as great (±58,000). Then the absolute number of cells in the S phase would be 1500 and for the cells in G2 and mitosis 700. In this phase, as already proposed, a steady state might sooner or later be attained and a psoriasis mitis, practically asymptomatic psoriasis, lesion might originate.

We then arrive at psoriasis vulgaris. The absolute number of cells has risen to 160,000 per mm^2, the number of cells in the S phase is 16,000 and those in G2 and mitosis 4800, demonstrating a marked, relative and absolute increase of cells in S phase. It seems likely that while the normal appearing skin of psoriasis is already in upheaval this might be much more the case for the clinical lesion.

We see now a new phenomenon occurring, the appearance of groups of nucleus-containing cells in the stratum corneum. Cox (1978) recently directed attention to the varying degrees of parakeratosis

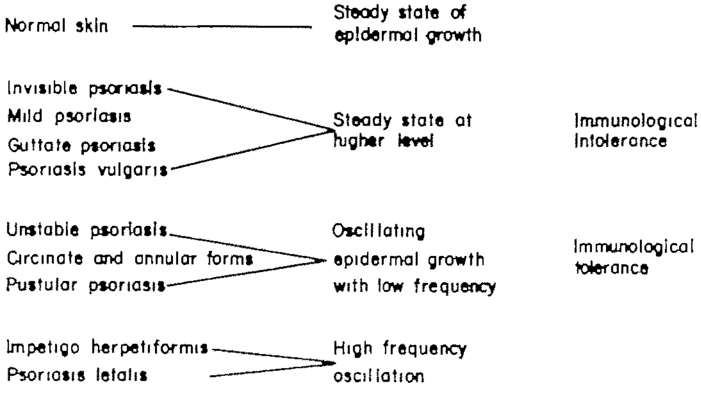
Normal skin
Steady state of epidermal growth
Invisible psoriasis
Mild psoriasis
Guttate psoriasis
Psoriasis vulgaris
Steady state at higher level
Immunological intolerance
Unstable psoriasis
Circinate and annular forms
Pustular psoriasis
Oscillating epidermal growth with low frequency
Immunological tolerance
Impetigo herpetiformis
Psoriasis letalis
High frequency oscillation

FIGURE 1. The gradual changes of clinical expression in psoriasis.

TABLE I.

	Total number/mm²	Ratio proliferating differentiating cells	Percentage and number of cells in different phases				
			G1*	S*	G2+M*	S†	G2+M
Normal skin	39,000	1:2	97·4 38,000	1·8 700	0·9 350	5·4	2·7
Normal appearing skin	60,000	1:1·5	96,3 58,000	2·5 1,500	1·2 700	6·3	3·0
Psoriasis lesion	160,000	1:1	87 139,000	10 16,000	3 4,800	20	6
Impetigo herpetiformis	200,000	1:1	75 150,000	20 40,000	5 10,000	40	10

* To the total cell number.
† To the proliferating cells.

in different parts of psoriatic scales. He sees it as evidence of a fluctuating epidermal growth activity that may be uniform throughout all parts of a single lesion. We should like to propose two mechanisms in order to explain this variability of parakeratosis, a key feature in our opinion of psoriasis. We have now to leave the path of hard facts and are coming into the fairyland of hypothesis, but we think this is necessary in order to get a direction for further investigation.

The bouts of parakeratosis can originate by interference of the cyclic activity of the basal cells by another factor with a different frequency, for example a hormone. This is a well-known phenomenon in engineering and in music, for instance, and results in what are called beat frequencies. A beat occurs at a frequency which is the difference between the two component frequencies. Thus a 10 Hz frequency mixed with an 11 Hz frequency will generate a new 1 Hz frequency. Thus from small, regular fluctuations in the levels of two hormones (inherent in negative feedback systems) cycles of far longer periodicities can arise. Such beat frequencies can also interact and we can now see that biological rhythms can be expected to be the norm, not just freakish occurrences. The new beat will interfere with the normal smoothly running asynchronic activities of the basal cells and might, at some points of the proliferating epithelium, bring a group of cells into phase. We might see the relative and absolute increase of cells in the S phase in the psoriasis lesion as such an event.

The small groups of synchronized cells would then interfere with each other, die slowly and become evicted by the surrounding, normally functioning cells. That this effect would be caused by a hormone is not so strange as the skin is intimately concerned with overall homeostatic processes such as heat and water conservation and salt retention. It is a relatively undifferentiated organ, as can be seen from the fact that it produces and modifies its own hormones, an activity delegated by most other organs to specific endocrine glands (Berliner, 1972).

We do not give the interacting hormone a name. In principle nearly any hormone might interfere with the destabilized psoriatic epithelium. Indeed all sorts of stimuli might induce 'stuttering' of the epidermis resulting in psoriasis vulgaris. However, in the more severe persistent forms a specific factor might play a role and we believe that we might designate a possible candidate for whose appointment as Great Disturber there are some arguments. This is the salt regulating corticoid aldosterone. Arguments are:

(1) It is the hormone regulating the salt balance, being active upon the sweat gland (Dobson &

Slegers, 1971; Sato, 1971). Its activity at the epidermal level is about ten times smaller than at the secreting part, but might just be enough for the unstable psoriasis skin.

(2) The lesions for psoriasis pustulosa originate in the vicinity of the epidermal part of the sweat gland (Rupec, 1970; Neumann & Hard, 1974). Psoriasis guttata seems also to originate from the sweat gland.

(3) There is a remarkable agreement between the histology of impetigo herpetiformis and the moulting process in amphibians. In both we see a massive necrosis of the outer layers of the stratum spinosum together with a marked proliferation of cells in the lower regions (Ling, 1972). By flow cytophotometry of these lower layers we find that the majority of all cells are in the S phase. That is to say, the inherent cycle of each cell (which is normally veiled by asynchronicity of the individual cycles) is disclosed to the outside world in the cycle of recurring attacks, which may vary from 2 to 4 weeks, and which are such a characteristic feature of impetigo herpetiformis. The hormone responsible for the elicitation of the moulting process is aldosterone (Smith, 1977).

We would like to propose that aldosterone, interfering at the epithelial part of the sweat gland, could, as a by-product, have some effect on the epithelial cells surrounding them. This might cause a block of these cells in the S phase, resulting in their synchronization and removal.

From the foregoing consideration it appears that we might suggest psoriasis is a dynamic disease, in the sense used by Mackay & Glass (1977). It would then be one of the human illnesses which may be associated with physiological control processes that are in one regime of dynamic behaviour for normal healthy people and in another regime for sick people.

Typically the physiological variable may be constant in the normal state, and oscillatory or chaotically varying in the pathological state. For psoriasis the regulated physiological variable would then be the thickness of the skin. All further disturbances might be considered the logical consequences of the primary chaotic situation. From this point of view it would appear attractive to consider impetigo herpetiformis as the archetypal, paradigmatical form of psoriasis (Mali, 1974). The inherent cyclic activity of the epithelial cells, normally covered by their asynchronicity, has become visible in this eruption. In a number of cases the influence of hormonal factors on the course of the disease can be established and even used for therapeutic purposes. Psoriasis vulgaris might then be considered as a minor variant of impetigo herpetiformis. Perhaps due to its connection with the HLA system, an immunological reaction on the altered epithelial cells, at the moment they become blocked, might seal off the altered cells and reduce the damage.

From this standpoint once more looking at psoriasis, we think the concept that psoriasis is a disease of the whole person, not only of the skin, is very tempting. A direct consequence would be to look for new pathways of treatment, not to the skin but towards the central (hypothalamic) regulating mechanisms.

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Beta-carotene treatment of erythropoietic protoporphyria

SIR, Corbett *et al.* (1977) reported on the relative ineffectiveness of beta-carotene in the treatment of erythropoietic protoporphyria (EPP). They used a placebo-controlled cross-over study design to repeat our studies; such a design has obvious merit. Unfortunately, there were serious defects in their study. The overwhelming defect was that they did not follow our therapeutic recommendations.

These authors stated (p. 657) that they used the dose recommended by us, namely 100 mg/day. In fact, we went to considerable pains to recommend an individualized dose reflecting each patient's needs, and found the range to be 15–180 mg/day. The dose was 'increased until the patient noted amelioration of photosensitivity' (Mathews-Roth *et al.*, 1974, p. 1001). We also recommended a minimum period of 3 months of treatment at doses giving blood levels of at least 800 μg/dl before it was safe to conclude that a given patient did not benefit from treatment. We made these dosage recommendations because we had found that although some patients and particularly children reported amelioration of photosensitivity with low doses of beta-carotene, the majority of adults need higher doses to receive symptomatic relief. Corbett *et al.* did not treat any children; their youngest patient was aged 19. Of the twenty patients 19 years and older treated in our study, fifteen needed to take 120 mg/day or more beta-carotene to receive benefit. Of the five who took less than this dose, two were judged to have doubtful benefit. Thus, individualized doses are necessary. These doses are higher in adults than in children, and, we have stressed, the necessary blood levels should be maintained for a long enough time to permit saturation of the appropriate tissue receptors of the photons that trigger the symptoms of the disease.

Figure 1 of Corbett *et al.* shows that the total period of ingestion of carotene for each group was less than 4 months, that blood levels above 800 μg/dl were maintained for less than a month, and that during part of one of the periods used for evaluation, blood carotene levels were below even 400 μg/dl. Therefore, the treatment used by these authors did not meet the criteria that we outlined, and certainly did not constitute a 'long term treatment'.

In addition, the choice of end-point by Corbett *et al.* was not a reproduction of our observations. They asked patients to note the total length of time spent per day in the sun, and to report symptoms of photosensitivity. We asked our patients to note the length of time they could stay in the sun before symptoms of photosensitivity developed, administering the same form of questionnaire before and during treatment with carotene (1974). This difference in end-points is important; it is not possible from the end-point used by Corbett *et al.* to determine whether a given patient could spend a longer symptom-free time in the sun, or whether the patient was responding to habit and previous conditioning based on the untreated disease. It should be noted that we have never claimed that with beta-carotene patients will spend longer periods of time in the sun (though many state that they do): our consistent finding has been that with treatment it takes a longer period of exposure before patients develop symptoms. We agree that objective rather than subjective measures of amelioration of symptoms are desirable. We have used amelioration of photosensitivity to measured doses of xenon arc light as reported in our papers (Mathews-Roth *et al.*, 1974, 1979), and we still believe that this approach is superior to any of the subjective evaluations such as those used by Corbett *et al.*, or by us. Our data would be suspect if they were not paralleled by the objective phototest data.

We present these comments with the hope that they may be of use to those interested in designing objective explorations of the use of beta-carotene treatment. We are happy to assist in the design of such studies in any way possible.

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SIR, Drs Mathews-Roth *et al.* suggest that, in our controlled trial of beta-carotene in EPP, we found no significant effect because the beta-carotene dose was too low and given for too brief a time. Of the patients they reported in 1974, only fourteen took over 120 mg beta-carotene daily, whereas twenty-three took 120 mg daily and as many as sixteen took 90 mg or less daily. One also gets the impression that the patients in the lowest dosage group improved as much as those on higher doses.

Our dosage, 100 mg/day, was in the mid-range and should have sufficed, since the serum beta-carotene levels were at least 500 μg/100 ml as against the 400 μg/100 ml suggested by Mathews-Roth and her colleagues. Again in their report of 1974 most of their patients noted benefits within 1–2 months of starting to take the beta-carotene. We are not persuaded by their arguments and believe that the treatment of our patients differed little from that in their 1974 study.

But our methods of assessment differ. We were trying to get away from appraising therapeutic results by relying on patients' memories of symptoms before and after treatment and by asking leading questions (see Findlay (1969) for a discussion of this). Our aim was to obtain data more directly and assess them statistically, hence the weekly posted diary cards (our Fig. 2) and list of symptoms from which a numerical 'symptom score' was derived to be correlated with patient exposure and sunshine hours. We discussed the shortcomings of our therapeutic trial in our report, but considered it of sufficient interest to publish because it was a first attempt to measure patients' symptoms and solar exposure objectively.

In our view the most serious objection to our trial is the small number of patients used; as we said, this is liable to lead to a Type II error, of incorrectly accepting a null hypothesis. We can avoid this statistical trap by a trial on a larger number of patients and this we hope to do when our U.K. Erythropoietic Protoporphyria Patient Register is fully established. Probably the U.K. is uniquely favourable for such a study in having both a National Health Service and a system whereby the Registrar General can inform research workers of the deaths of patients who are being followed up.

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Juvenile plantar dermatosis

SIR, Juvenile plantar dermatosis (JPD) is one of many names given to a characteristic dermatosis of infancy and childhood localized to the distal soles including the toes, particularly the big toe, but sparing the toe spaces. MacKie & Husain (1976) described 102 children with the condition and there are many other reports both earlier and later in the literature. I should like to comment on the excellent study by Shrank (1979) on JPD in which he indicates how modern shoe materials initiate miliaria. I can accept his suggestion that the anhidrosis in JPD may be due to miliaria but ridge flattening and absence of visible pores are, in my opinion, more likely a consequence of the dry skin rather than an outward sign of oedema of the keratinocytes. It is perhaps relevant that in pompholyx, despite both intraepidermal vesicles and spongiosis, external ridge appearances are preserved although the epidermis is stretched. Flattening of epidermal ridges over soles is a common finding in individuals with dry skin and in atopic dermatitis even in the absence of active eczema in the area.

I believe that JPD not only describes a true localized atopic dermatitis but also a frictional contact dermatitis to which atopics are prone but which may occur in susceptible non-atopics (Verbov, 1976, 1978a, b). My series dating from 1973 has reached seventy-two (M35, F37) and thirty-three (46%) of these have a personal or family history of atopy. The criteria for a diagnosis of atopy are a personal or family history of asthma, hay fever, perennial rhinorrhoea, or typical eczema of atopic type. Using similar criteria Millard & Gould (1977) and Jepsen (1979) also found a high incidence of atopy.

Surprisingly none of Shrank's patients was atopic and it is most interesting that his skin biopsies, although showing features of miliaria, did not show dermatitis although van Dijk *et al.* (1978) did demonstrate a dermatitis histology.

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Malignant atrophic papulosis

SIR, Professor Degos' excellent review of malignant atrophic papulosis (*British Journal of Dermatology* (1979) **100**, 213) which I enjoyed reading invites a comment. The entity now known as Degos' disease was recognized and described in 1941 by Köhlmeier (*Archives of Dermatology and Syphilology* (1941) **181**, 783) who gave a detailed and well-documented description of the skin and gastrointestinal including the histopathological features of this disease. One year later, in 1942, Degos *et al.* independently reported on the same condition as 'Dermatite papulosquameuse atrophiante' (*Bulletin de la Société Français de dermatologie et syphiligraphie* (1942) **49**, 148). It is a pity that more credit is nót generally given to Köhlmeier, although he may not have considered this a separate entity.

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SIR, It is quite true that Köhlmeier published in 1941 a case entitled 'Thrombo-angiitis obliterans' with cutaneous lesions absolutely comparable to those of malignant atrophic papulosis and with a fatal outcome due to peritonitis. This author had considered the case as an unusual complication of Buerger's disease, and did not recognize that he was dealing with an autonomous entity. I did mention the case of Köhlmeier on page 32 of my review in *British Journal of Dermatology*, and in the bibliography.

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British Journal of Dermatology (1979) **101,** 735.

Book Reviews

Investigative Techniques in Dermatology. Edited by R. MARKS (1979) Oxford: Blackwell Scientific Publications. Pp. 275, B/W illustrated. Price £14.50.

The book comprises nineteen chapters each of which is a contribution from an expert with practical experience in one particular branch of investigative medicine of interest to dermatologists. The book was based on a series of lectures given in Cardiff and so it is hardly surprising that five of the contributors work there; the rest, with one exception, work in other parts of Britain and many of the names are household words.

The fact that there is not really a unifying theme running through the book (apart from the very broad one of investigative techniques) is almost inevitable when one considers that an editor is to a great extent dependent upon the special interests of the colleagues and friends he asks to contribute. The subjects vary for example from the very clinical patch testing to the laboratory details of porphyrin estimations and from the theory behind clinical trial design to the practice of electron microscopy. Some techniques like skin lipid measurements are special to dermatology while others like tests of lymphocyte function are of wider interest.

All the chapters are well written and attractively set out and in places there is even a touch of humour. The editor says he has avoided the cookery book approach—first catch your hairless mouse?—but good practical advice is given in most sections so that aspiring investigators will buy the book and go into the lab or clinic with it in hand; those more at home in the armchair could also do worse than buy a copy to read.

JANET MARKS

Allergy in Children. A Guide to Practical Management. By JAN A. KUZEMKO (1978). Tunbridge Wells: Pitman Medical. Pp. 144. Price £6.00.

Most dermatologists have realized that many of their clinical problems have an immunological basis or component. Whereas clinical immunology is considered a respectable term, 'allergy' is still assigned by many medical specialists to the subjects which have not yet attained full respectability. The teaching of this important subject is sadly neglected at most medical schools in this country and the D.H.S.S. has so far shown reluctance to recognize allergy and clinical immunology as a separate speciality although it has much to offer to paediatricians, dermatologists, general physicians, clinical pharmacologists, transplant surgeons and to many other clinical disciplines.

Since allergic phenomena are most obvious in respiratory tract and numerous childhood disorders, it is not surprising that enthusiastic members of these specialities have done much to further clinical knowledge in this field. However, the complexities of modern immunological techniques make essential sophisticated laboratory support, which is particularly scanty in this country in contrast to others such as the U.S.A. and Scandinavia.

In this small monograph Dr Kuzemko has made a brave attempt to bring together the disorders of the alimentary tract, the skin, the respiratory tract, the ears, the eyes and the nervous system which he considers would benefit from an immunological approach. He has also managed to include a chapter on drug allergy, on allergic reactions to insect bites and stings and several hundred most useful references—all within 137 pages.

The author has tried to incorporate in this little book a remarkable amount of clinical medicine and to guide the reader to further sources of information.

Apparently 'this short text is intended for the doctor caring for children'. It is a brave attempt to give much needed guidance. It would be churlish to criticize controversial details, out of date concepts, or the omission of important recent work, even allowing for the delay in publishing the manuscript.

The wealth of information, common sense and the author's enthusiasm make this a commendable book, but its readers should not assume that it will absolve them from keeping up a close acquaintance with *Clinical Allergy* and the *Lancet*.

G.HOLTI

Treatment of Skin Diseases. A Manual. By CHARLES SHEARD (1978) Chicago: Year Book Medical Publishers. Price £16.25.

This book illustrates the treatment of skin diseases in competitive 'office' practice. 'Using aqueous gentian violet is a good way to lose a patient but it is an excellent treatment . . .'; 'The attitude that there is something inherently unscientific in the application of successful empiricism should be abandoned'; 'If it works use it'.

Previous scientific training however is mandatory before adopting some of the recommendations, such as the local use of benzocaine, sulphonamides, mercurials, antibiotics and salicylic acid. Dithranol (anthralin) is not mentioned in the rather short section on psoriasis. Much wisdom is unfortunately lost to the European reader from the use of American proprietary names.

The advice on how to write a prescription in front of a patient is a must for the young part-time consultant as well as the insistence on frequent follow-up until 'cured'. In conclusion: 'Besides the stamina to stand on one's own feet for 16 hours a day, the effective clinician must be able to manage clinically difficult problems . . . the patient must see his doctor's concern for his personal problem and sense that something is being done to relieve him'.

R.H.SEVILLE

UV-A. Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet. By JOHN A. PARRISH, R. ROX ANDERSON, FREDERICK URBACH and DONALD PITTS (1979). New York: Plenum Press (distributed in U.K. by John Wiley, Chichester). Pp. 262, B/W illustrated. Price £15.00.

Following a short introduction (Chapter 1) dealing with the ultraviolet component of the solar spectrum and which summarizes the more important aspects of the effect of the longer UV wavelengths, the authors describe sources of UV-A irradiation, both natural and artificial (Chapter 2), and detail the methods which can be used to select wavebands where the energy output and purity of emission can be accurately measured (Chapter 3). These chapters are of particular interest to the investigator who requires suitable phototesting equipment, and the authors rightly stress the importance of the predictability of this purity of emission since contamination of the UV-A irradiation by even small amounts of the more active shorter UV-B wavelengths must be avoided. Consideration of the optical properties of the skin (Chapter 4) is necessary in the understanding of the relative importance of the absorption of the UV wavelengths by the different layers and structures and how this will vary with epidermal thickness, melanin pigmentation production, etc. This is particularly relevant to any study of skin ageing and of the action of UV-A in phototherapy and in photochemotherapy. The second half of this chapter deals with the UV optics of the eye. The effects of ultraviolet radiation on micro-organisms and animal cells are dealt with in Chapter 5, and following a short review of the known effects of UV on cells and the various repair mechanisms involved, the more specific effect of UV-A is discussed, it being stressed once again that this should not be considered in isolation from the UV-B and the other parts of the solar spectrum. They rightly mention that interpretation of past reports of the effect of UV-A is difficult on account of the unreliability of the irradiation sources used, and that one must look to future studies to provide more of the answers. Until recently the study of the immediate and short-term biological effects of UV exposure on the skin (Chapter 6) has concentrated on the effect of UV-B, and it is therefore important to be reminded of the part played by the UV-A component in the production of erythema and pigmentation and of the processes involved. This leads naturally to a discussion of the application of current knowledge of the effects of ultraviolet radiation on human skin to the clinical problems experienced in dealing with the adverse cutaneous reactions to ultraviolet and particularly to UV-A (Chapter 7); and of the part played by these longer wavelengths in the aetiology, natural history, and treatment of the photodermatoses. The various environmental and social changes of recent years, which have increased the opportunity for exposure of the skin to ultraviolet radiation, makes the comprehensive review of skin ageing and carcinogenesis due to UV irradiation (Chapter 8) particularly important and here the mechanisms involved and the relative importance of the UV-B and UV-A component in photocarcinogenesis and the ageing process are discussed. The book ends with separate chapters dealing with the effect of ultraviolet on the eyes; the use of UV-A in phototherapy and photochemotherapy; and lastly, safety measures and protection which can be used to minimize the effect of UV exposure. At the end of each chapter there is a selected list of references.

This is a readable and comprehensive study of the effects of ultraviolet radiation. The authors have succeeded in their aim of providing a general view of the effect of ultraviolet, and pointing out where relevant the particular importance of the longer wavelength UV-A, and in providing suggestions and help for those who wish to study this aspect, and in particular in the fundamental effects of light on the skin, in photocarcinogenesis and skin ageing, and in future developments in phototherapy and photochemotherapy. It is unfortunate therefore that Chapter 7, which most clinicians will turn to first, deals inadequately with what is the major interest of many of us, i.e. cutaneous photosensitivity. Although this book is obviously intended for a wider readership than simply that of the dermatologist, the practising clinician will look to such a monograph to provide him with a reasoned and objective assessment which draws attention to the more important effects of ultraviolet on the human skin. He would hope then to apply the knowledge gained in such a way that allows him to investigate, treat, and advise patients with common dermatological problems in which photosensitivity is a factor.

Perhaps in the next edition of this useful monograph the authors will consider rewriting Chapter 7.

W.FRAIN-BELL

British Journal of Dermatology (1979) **101**, 738.

Annual General Meeting

Annual Meeting of the British Association of Dermatologists

The 59th Annual Meeting of the British Association of Dermatologists was held in London from 4 to 7 July 1979 under the Presidency of Dr Stephen Gold; the Local Secretary was Dr K.V. Sanderson. The number of members and guests attending was 332 and in addition several wives were present. The official guests this year were from the Netherlands Dermatological Society and we were pleased to welcome ten members, some of whom were accompanied by their wives.

The Annual General Meeting was held on Wednesday afternoon, 4 July as it had been at the 58th Annual Meeting in Nottingham and there was considerable discussion about the future training of dermatologists and the staffing of consultant posts.

The scientific presentations were given at the Royal College of Physicians, Regents Park, and the accommodation in the lecture theatre was over-tested by the numbers attending. Twenty-five papers were presented and the three guest lecturers were Dr Martin Beare, Dr Richard Dobson and Dr Peter Richards.

The Librarian at the Royal College of Physicians arranged an exhibition of books of dermatological interest which was greatly admired.

Because of the large numbers of people attending, the official reception was held partly at the Royal Hospital Chelsea and partly at Chandos House.

The social events included a very successful 1-day visit to Leeds Castle on Thursday, 5 July, afternoon visits to Hampton Court and the Physic Garden at Chelsea on 6 July followed that evening by the annual dinner at the Savoy Hotel when 289 members and guests attended.

The Dowling McCaw Golf Cup was won by Dr J.A. Savin, Dr Paul August and Dr E.N.M. Johnson. The Bowers/Sneddon Sailing Trophy was won by Dr Peter Bowers.

There were eleven excellent entries in the scientific poster exhibition and the Bristol Cup was awarded to Dr Fenella Wojnarowska and her two collaborators.

The Clinical Meeting was held at St George's Hospital Medical School, Tooting on Saturday, 7 July where, in addition to the thirty-four cases, there was an outstanding exhibition of moulages and microscopic drawings and paintings by Miss Alice Gretener, together with a display of old dermatological books and atlases.

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News and Notices

5th International Clinically Oriented Symposium of the E.S.D.R. on The Biology of the Histiocyte and its Role in Cutaneous Pathology

This Symposium will be held in Catania, Sicily, Italy, on 11–12 January 1980, at the Auditorium of the Grand Hotel Baia Verde.

The official language will be English; simultaneous translation into Italian will be available.

Enquiries concerning the scientific organization of the Symposium should be sent to Dr Antonio Garcovich, Clinica Dermatologica, Universita del Sacro Cuore, Largo A. Gemelli 8, 00168 Rome, Italy.

Information concerning accommodation, social and Ladies' programme, may be obtained from American Express Co. S.A.I., Conventions Service, Piazza Miganelli 4, 00187 Rome, Italy.

Dermatopathology Symposium, Rome, 3–4 May 1980

Under the sponsorship and organization of the Foundation for Research in Dermatology an intensive 2 day course entitled 'Histologic diagnosis of inflammatory skin diseases: a method by pattern analysis' will be given by Dr A. Bernard Ackerman, Professor of Dermatology and Pathology and Director of Dermatopathology, New York University School of Medicine, at the Catholic University, Policlinico Gemelli, in Rome, Italy, on Saturday and Sunday, 3–4 May 1980.

A method will be taught for making specific histological diagnoses of inflammatory diseases in the skin by recognition of nine distinctive patterns, namely, superficial perivascular dermatitis, superficial and deep perivascular dermatitis, vasculitis, nodular and diffuse dermatitis, subepidermal vesicular dermatitis, folliculitis and perifolliculitis, fibrosing dermatitis, and panniculitis.

Enrolment is limited, and enquiries should be directed to: Ferdinando Serri, Professor and Chairman, Dept. of Dermatology, Università Cattolica, Largo Gemelli 8, 00168 Rome, Italy.

Dermatopathology Symposium, Vienna, May 1980

An international dermatopathology symposium coorganized by A.B. Ackerman (New York University), K. Konrad, W. Gebhart, (University of Vienna) and H. Kerl (University of Graz) will be held on 8 May 1980 in Vienna, Austria. Anyone wishing to contribute or to attend should contact Dozent Dr K. Konrad, Department of Dermatology I, University of Vienna, A-1090 Vienna, Austria.

7th European Meeting of the Society of Cutaneous Ultrastructure Research

The 7th European Meeting of the Society of Cutaneous Ultrastructure Research will be held in Vienna, Austria, 9–10 May 1980. Anyone wishing to contribute or to attend should contact Dozent Dr. W. Gebhart, Department of Dermatology II, University of Vienna, A-1090 Vienna, Austria.

2nd International Conference of the European Society for Comparative Skin Biology

The 2nd International Conference of the European Society for Comparative Skin Biology will be held at the August Krogh Institute, University of Copenhagen on 25–28 August 1980.

Papers will be accepted on skin structure and function, transport and barrier function, dermo-epidermal interactions, proliferation, differentiation and cuticular biology.

Details of registration may be obtained from Dr P.E. Budtz, Zoophysiological Laboratory A, The August Krogh Institute, 13, Universitetsparken, DK-2100 Copenhagen Ø, Denmark.

Dermatopathology Foundation Course

A Dermatopathology Foundation Course in Gross and Microscopic Pathology of the Skin will be held at the Sheraton-Deauville Hotel, Atlantic City, New Jersey, U.S.A. on 16–20 June 1980 (40 Hours Credit—A.M.A.—Category—1).

Further information may be obtained from Dermatopathology Foundation, PO Box 377, Canton, MA 02021, U.S.A.

B.A.D. Travelling Fellowships

Applications are invited for the short-term Travelling Fellowships awarded by the British Association of Dermatologists annually. Further information and application forms can be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES.

Dowling Travelling Fellowship

Applications are invited for the Dowling Travelling Fellowship in Dermatology which is awarded triennially. Applicants must hold a higher medical qualification and the aim of the Fellowship is to encourage evident merit in junior physicians working in the field of Dermatology. Further details can be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES. The closing date for the receipt of applications is 30 January 1980.

John Milne Travelling Fellowship

The recipients of the first John Milne Travelling Fellowship are Mrs Irene Hunter and Dr Raymond Foulton. Both these individuals will receive finance to facilitate their attendance at meetings in the continent during 1980.

The next John Milne Travelling Fellowship will be awarded in the autumn of 1981.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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